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**WO 00/75187 A1**

(54) Title: AN INTEGRIN HETERODIMER AND AN ALPHA SUBUNIT THEREOF

(57) Abstract: A recombinant or isolated integrin heterodimer comprising a novel subunit  $\alpha 11$  in association with a subunit  $\beta$  is described. The integrin or the subunit  $\alpha 11$  can be used as marker or target of all types of cells. The integrin or subunit  $\alpha 11$  thereof can be used as marker or target in different physiological or therapeutic methods. They can also be used as active ingredients in pharmaceutical compositions and vaccines.

AN INTEGRIN HETERODIMER AND AN ALPHA SUBUNIT THEREOFFIELD OF THE INVENTION

The present invention relates to a recombinant or isolated integrin heterodimer comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , the subunit  $\alpha 11$  thereof, homologues and  
5 fragments of said integrin and of said subunit  $\alpha 11$ , processes of producing the same, polynucleotides and oligonucleotides encoding the same, vectors and cells comprising the same, binding entities binding specifically to binding sites of the same, and the use of  
10 the same.

BACKGROUND OF THE INVENTION

Integrins are heterodimers composed of non-covalently associated  $\alpha$ - and  $\beta$ -chains which connect cells to the extracellular matrix or to other cells (1). In addition  
15 to acting as mechanical links between the cytoskeleton and extracellular ligands, integrins are signal transducing receptors which influence processes such as cell proliferation, cell migration and cell differentiation (2-4). Integrins can be grouped into subfamilies based on  
20 shared  $\beta$ -chains, shared ligand binding properties, or shared structural features of the  $\alpha$ -chains. Currently 17  $\alpha$ -chains and 8  $\beta$ -chains have been identified (5). Of the subfamilies with shared  $\beta$ -chains, the  $\beta 1$  subfamily has the most members. To date, 11 integrin  $\alpha$ -chains associated with the  $\beta 1$ -chain have been identified and characterized,  $\alpha 1$ - $\alpha 10$  and  $\alpha v$  (5).  
25

Several integrins bind the sequence RGD in their respective ligands (1). Of those integrins identified so far,  $\alpha 4$ -,  $\alpha 5$ -,  $\alpha 6$ -,  $\alpha IIb$ - and  $\alpha v$ -chains form heterodimers  
30 that mediate RGD-dependent interactions. The ligands containing RGD are generally found in the interstitial type of extracellular matrix. Major non-RGD dependent ligands include various collagen and laminin isoforms. Although both collagens and laminins contain the RGD

sequence in their primary sequences, these RGD sequences are cryptic (6-9) and normally not accessible to cells in the native proteins, but they may be exposed during growth and reorganization events of the extracellular matrix.

Another subdivision of integrins can be made based on structural similarities of the  $\alpha$ -chains. A number of integrins contain an extracellular I-domain (10,11) which is homologous to collagen binding A-domains present in von Willebrand factor (12). The I-domain constitutes an inserted domain of approximately 200 amino acids which is present in 8 known integrins ( $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 10$ ,  $\alpha L$ ,  $\alpha M$ ,  $\alpha X$ ,  $\alpha D$  and  $\alpha E$ ) (5,10). Structural analysis of integrin I-domains crystallized in the presence of  $Mg^{2+}$  have revealed the presence of a characteristic "MIDAS" (metal ion dependent adhesion site) motif, shown to be critical for ligand binding (13). Integrin  $\alpha$ -chains containing the I-domain are not cleaved into heavy and light chains, although the rat  $\alpha 1$  chain possesses a proteolytic cleavage site near the membrane spanning region (14,15). For I-domain integrins the principal ligand binding sites are found within the I-domain (10). Known ligands for I-domains found within the  $\beta 1$  integrin subfamily include laminins and collagens ( $\alpha 1\beta 1$  and  $\alpha 2\beta 1$  integrins) (16-19), and Echo-virus ( $\alpha 2\beta 1$  integrin) (20).

Structure comparisons have suggested that integrins fold into a so-called 7-bladed  $\beta$ -propeller structure which forms one globular domain with the ligand binding region on the upper surface (21). The I-domain is inserted between blade 2 and 3 in this propeller and divalent cation binding sites are located on the lower surface in blades 4-7 (22,23). Studies of  $\beta 2$  integrins have revealed that proper folding of the  $\beta 2$ -chain is dependent on the presence of the  $\alpha L$ -chain but that the I-domain folds independently of other structural elements in the  $\alpha$ - and  $\beta$ -chains (24). In integrin  $\alpha$ -chains, a less conserved stalk region separates the predicted  $\beta$ -pro-

pellier from the short transmembrane region. This stalk region is possibly involved in transducing conformational changes between the extracellular and intracellular regions, as well as mediating protein-protein interactions. Although integrins take part in cell signalling events, the cytoplasmic tail is short and lacks enzymatic activity. The sequence GFFKR is conserved in a majority of integrin  $\alpha$ -subunits cytoplasmic tails and has been shown to be important for calreticulin binding (25).

Cellular interactions with the extracellular matrix during muscle formation and in muscular dystrophy have received increased interest during the past years. In the early 1960's a mutant was described in *Drosophila* which was characterized by the detachment of muscles from their attachment points at the time of the first embryonic muscle contraction, causing the embryos to assume a spheroid shape (26). The mapping of the molecular defect in the lethal myspheroid mutant in 1988 to an integrin  $\beta$ -chain (27), was the first evidence for a role of integrins in maintaining muscle integrity. More recently, refined analysis of *Drosophila* mutants have indicated distinct roles for integrins in muscle endpoint attachments and sarcomere structure (28). The *Drosophila* integrins are all cleaved  $\alpha$ -chains and share many features with vertebrate integrins such as the ability to cluster into focal contacts (29).

The finding that inactivation of the  $\alpha 7$  integrin gene in mouse (30), as well as mutations in the human ITGA7 gene (31), both cause muscular dystrophy affecting mainly muscle attachment points, indicates a striking conservation of integrin function during evolution. Of the 11 members of the  $\beta 1$  subfamily,  $\alpha 7$  exists as a major integrin  $\alpha$ -chain (32,33) associated with the  $\beta 1 D$  integrin chain in the adult skeletal muscle sarcolemma (34). Intriguingly, mutations in the basement membrane protein laminin  $\alpha 2$ -chain (35-37) cause a more severe disease than that observed for the laminin receptor integrin  $\alpha 7 \beta 1$

(30). This indicates that other receptors for laminins exist in muscle.

A novel integrin has recently been identified on cultured human fetal muscle cells (38). The present invention is related to, inter alia, the cloning and characterization of this novel I-domain containing,  $\beta 1$ -associated integrin chain, which is expressed in muscle tissues.

#### SUMMARY OF THE INVENTION

10 The full-length cDNA for this integrin subunit,  $\alpha 11$ , has now been isolated. The open reading frame of the cDNA encodes a precursor of 1188 amino acids. The predicted mature protein of 1166 amino acids contains 7 conserved FG-GAP repeats, an I-domain with a MIDAS motif, a short  
15 transmembrane region and a unique cytoplasmic domain of 24 amino acids containing the sequence GFFRS.  $\alpha 11$ , like other I-domain integrins, lacks a dibasic cleavage site for generation of a heavy and a light chain, and contains three potential divalent cation binding sites in re-  
20 peats 5-7. The presence of 22 inserted amino acids in the extracellular stalk portion (amino acids 804-826) distinguishes the  $\alpha 11$  integrin sequence from other integrin  $\alpha$ -chains. Amino acid sequence comparisons reveal the highest identity of 42% with  $\alpha 10$  integrin chain. Immuno-  
25 precipitation with antibodies to  $\alpha 11$  integrin captures a 145 kD protein, distinctly larger than the 140 kD  $\alpha 2$  integrin chain when analyzed by SDS-PAGE under non-reducing conditions. Fluorescence in situ hybridization maps the integrin  $\alpha 11$  gene to chromosome 15q23, in the  
30 vicinity of an identified locus for Bardet-Biedl syndrome. Based on Northern blotting integrin  $\alpha 11$  mRNA levels are high in adult human uterus and in heart, and intermediate in skeletal muscle and some other tissues tested. During in vitro myogenic differentiation,  $\alpha 11$   
35 mRNA and protein are up-regulated. Studies of ligand binding properties show that  $\alpha 11\beta 1$  binds collagen type

I Sepharose and cultured muscle cells localize  $\alpha 11\beta 1$  into focal contacts on collagen type I.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates in its different aspects to the following:

A recombinant or isolated integrin subunit  $\alpha 11$  comprising essentially the amino acid sequence shown in SEQ ID No. 1, or homologues or fragments thereof.

The invention also encompasses integrin homologues of said integrin, isolated from other species, such as bovine integrin heterodimer comprising a subunit  $\alpha 11$  in association with a subunit  $\beta$ , preferably  $\beta 1$ , as well as homologues isolated from other types of human cells or from cells originating from other species.

The term "homologues" in the context of the present invention is meant to imply proteins of a common evolutionary origin, having identical or similar functions, specifically requiring evidence based on gene structure and not merely a similarity of protein structure.

The invention also encompasses a process of producing a recombinant integrin subunit  $\alpha 11$  comprising essentially the amino acid sequence shown in SEQ ID No. 1, or homologues or fragments thereof, which process comprises the steps of

- a) isolating a polynucleotide comprising a nucleotide sequence coding for an integrin subunit  $\alpha 11$ , or homologues or fragments thereof,
- b) constructing an expression vector comprising the isolated polynucleotide,
- c) transforming a host cell with said expression vector,
- d) culturing said transformed host cell in a culture medium under conditions suitable for expression of integrin subunit  $\alpha 11$ , or homologues or fragments thereof, in said transformed host cell, and, optionally,

e) isolating the integrin subunit  $\alpha 11$ , or homologues or fragments thereof, from said transformed host cell or said culture medium. The transformation can be performed in vitro, in situ or in vivo.

5 In further aspects, the invention encompasses:

- A process of providing an integrin subunit  $\alpha 11$ , or homologues or fragments thereof, whereby said subunit is isolated from a cell in which it is naturally present.

10 - An isolated polynucleotide comprising a nucleotide coding for said integrin subunit  $\alpha 11$ , or for homologues or fragments thereof, which polynucleotide comprises essentially the nucleotide sequence shown in SEQ ID No. 1 or suitable parts thereof.

15 - An isolated polynucleotide or oligonucleotide which hybridises to a polynucleotide or oligonucleotide encoding said integrin subunit  $\alpha 11$  or homologues or fragments thereof, wherein said isolated polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit  $\alpha 10$ .

20 - A vector comprising a polynucleotide or oligonucleotide coding for said integrin subunit  $\alpha 11$ , or homologues or fragments thereof, which polynucleotide or oligonucleotide comprises the nucleotide sequence shown in SEQ ID No. 1 or parts thereof.

25 - A vector comprising a polynucleotide or oligonucleotide which hybridises to a DNA or RNA encoding an integrin subunit  $\alpha 11$  or homologues or fragments thereof, wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit  $\alpha 10$ .

30 - A cell containing the vector as defined above.

- A cell generated during the process as defined above, in which a polynucleotide or oligonucleotide coding for said integrin subunit  $\alpha 11$ , or homologues or fragments thereof, which polynucleotide or oligonucleotide comprises essentially the nucleotide sequence shown

in SEQ ID No. 1 or parts thereof, has been stably integrated in the cell genome.

- Binding sites of the amino acid sequence of the integrin subunit  $\alpha 11$ , or of homologues or fragments thereof, said binding sites having the capability of binding specifically to entities chosen from the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, polyclonal and monoclonal antibodies, and fragments thereof.

- Binding entities having the capability of binding specifically to integrin subunit  $\alpha 11$  comprising the amino acid sequence of SEQ ID No. 1 or to homologues or fragments thereof, preferably chosen from the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, polyclonal and monoclonal antibodies, and fragments thereof.

- A recombinant or isolated integrin heterodimer comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , in which the subunit  $\alpha 11$  comprises essentially the amino acid sequence shown in SEQ ID No. 1, or homologues and fragments thereof. Said subunit  $\beta$  is preferably  $\beta 1$ .

- A process of producing a recombinant integrin heterodimer comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , in which the subunit  $\alpha 11$  comprises essentially the amino acid sequence shown in SEQ ID No. 1, or homologues or fragments thereof, which process comprises the steps of

a) isolating one polynucleotide comprising a nucleotide sequence coding for a subunit  $\alpha 11$  of an integrin heterodimer and, optionally, another polynucleotide comprising a nucleotide sequence coding for a subunit  $\beta$  of an integrin heterodimer, or polynucleotides or oligonucleotides coding for homologues or fragments thereof having similar biological activity,

b) constructing an expression vector comprising said isolated polynucleotide coding for said subunit  $\alpha 11$  optionally in combination with an expression vector com-



prising said isolated nucleotide coding for said subunit  $\beta$ ,

c) transforming a host cell with said expression vector or vectors, which transformation may be performed  
5 in vitro, in situ or in vivo,

d) culturing said transformed host cell in a culture medium under conditions suitable for expression of an integrin heterodimer comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , or homologues or fragments thereof, in said  
10 transformed host cell, and, optionally,

e) isolating the integrin heterodimer comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , or homologues or fragments thereof, or the  $\alpha 11$  subunit thereof from said transformed host cell or said culture medium.

15 - A process of providing an integrin heterodimer comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , or homologues or fragments thereof having similar biological activity, whereby said integrin heterodimer is isolated from a cell in which it is naturally present.

20 - A cell containing

i) a first vector, said first vector comprising a polynucleotide or oligonucleotide coding for a subunit  $\alpha 11$  of an integrin heterodimer, or for homologues or parts thereof, which polynucleotide or oligonucleotide  
25 comprises essentially the nucleotide sequence shown in SEQ ID No. 1 or parts thereof, and

ii) a second vector, said second vector comprising a polynucleotide or oligonucleotide coding for a subunit  $\beta$  of an integrin heterodimer, or for homologues or fragments thereof.  
30

- Binding sites of an integrin heterodimer as defined above, or of homologues or fragments thereof, said binding sites having the capability of binding specifically to entities chosen among the group  
35 comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, polyclonal and monoclonal antibodies, and fragments thereof.

- Binding entities having the capability of binding specifically to said integrin heterodimer, or to homologues or fragments thereof, or a subunit  $\alpha 11$  thereof. Said subunit  $\beta$  is preferably  $\beta 1$ . The binding entities are preferably chosen among the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, and fragments thereof.

- A fragment of the integrin subunit  $\alpha 11$ , which fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, especially a peptide comprising essentially the amino acid sequence KLGFFRSARRRRREPGLDPTPKVLE, of the I-domain, especially a peptide comprising essentially the amino acid sequence from about amino acid No. 159 to about amino acid No. 355 of SEQ ID No. 1, and the extracellular extension region, especially a peptide comprising essentially the amino acid sequence from about amino acid No. 804 to about amino acid No. 826 of SEQ ID No. 1.

- A method of producing a fragment of the integrin subunit  $\alpha 11$  as defined above, which method comprises a sequential addition of amino acids. This method comprises adding and removing protective groups in a manner known by the man skilled in the art.

- A polynucleotide or oligonucleotide coding for a fragment of the integrin subunit  $\alpha 11$  as defined above.

- Binding sites of a fragment as defined above, said binding sites having the capability of binding specifically to entities chosen from the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, and fragments thereof.

- Binding entities having the capability of binding specifically to a fragment as defined of the human integrin subunit  $\alpha 11$  as defined above. Preferably, said binding entities are chosen from the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, and fragments thereof.

- A process of using an integrin subunit  $\alpha 11$  comprising essentially the amino acid sequence shown in SEQ ID No. 1 or an integrin heterodimer comprising said subunit  $\alpha 11$  and a subunit  $\beta$ , or a homologue or fragment of said integrin or subunit, as a marker or target molecule of cells or tissues expressing said integrin subunit  $\alpha 11$ , which cells or tissues are of animal including human origin. Especially, said subunit  $\beta$  is  $\beta 1$ .

In embodiments of this process, said fragment is a peptide chosen from the above defined group.

In one embodiment of said process, the cells are chosen from the group comprising fibroblasts, muscle cells, chondrocytes, osteoblasts, mesenchymally derived cells and stem cells.

Especially, said process is used during pathological conditions involving said subunit  $\alpha 11$ . Said pathological conditions comprise in one embodiment damage of muscles, muscle dystrophy, fibrosis or wound healing. In another embodiment, said pathological conditions comprise damage of cartilage and/or bone, or cartilage and/or bone diseases. In a still further embodiment, said pathological conditions comprise trauma, rheumatoid arthritis, osteoarthritis or osteoporosis.

In a further embodiment, said process is a process for detecting the formation of cartilage during embryonic development, or for detecting physiological or therapeutic reparation of cartilage and/or muscle, or for selection and analysis, or for sorting, isolating or purification of chondrocytes and/or muscle cells, or for detecting regeneration of cartilage or chondrocytes during transplantation of cartilage or chondrocytes, respectively, or of muscle or muscle cells during transplantation of muscle or muscle cells, respectively, or for studies of differentiation of chondrocytes or muscle cells.

Said process may be and *in vitro*, an *in situ* or an *in vivo* process.

- A process of using binding entities having the capability of binding specifically to binding sites of an integrin subunit  $\alpha 11$  as defined above, or of an integrin heterodimer comprising said subunit  $\alpha 11$  and a subunit  $\beta$ , or to homologues or fragments thereof, as markers or target molecules of cells or tissues expressing said integrin subunit  $\alpha 11$ , which cells or tissues are of animal including human origin. Especially, said subunit  $\beta$  is  $\beta 1$ .

In embodiments of this process, said fragment is as defined above.

In one embodiment, said process is a process for detecting the presence of an integrin subunit  $\alpha 11$  comprising the amino acid sequence shown in SEQ ID No. 1, or of an integrin heterodimer comprising said subunit  $\alpha 11$  and a subunit  $\beta$ , or of homologues or fragments thereof.

Furthermore, embodiments of this process encompass similar embodiments as defined above in connection with the process of using the integrin subunit  $\alpha 11$  as a marker or target molecule.

- A process for detecting the presence of an integrin subunit  $\alpha 11$ , or of a homologue or fragment of said integrin subunit, as defined above, on cells, whereby a polynucleotide or oligonucleotide chosen from the group comprising essentially a polynucleotide or oligonucleotide as shown in SEQ ID No. 1 is used as a marker under hybridisation conditions, wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit  $\alpha 10$ . Said cells may be chosen from the group comprising muscle cells.

In embodiments of this process, said fragment is as defined above.

Furthermore, embodiments of this process encompass similar embodiments as defined above in connection with the process of using the integrin subunit  $\alpha 11$  as a marker or target molecule.

- A pharmaceutical composition comprising as an active ingredient a pharmaceutical agent or an antibody which is capable of using an integrin heterodimer comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , or the subunit  $\alpha 11$  thereof, or a homologue or fragment of said integrin or subunit  $\alpha 11$ , as a target molecule.

- A pharmaceutical composition comprising as an active ingredient a pharmaceutical agent or an antibody which is capable of stimulating cell surface expression or activation of an integrin heterodimer comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , or the subunit  $\alpha 11$  thereof, or homologues or fragments of said integrin or subunit  $\alpha 11$ . In one embodiment, said composition is for use in stimulating, inhibiting or blocking the formation of muscles, cartilage, bone or blood vessels.

- A vaccine comprising as an active ingredient at least one member of the group comprising an integrin heterodimer, which heterodimer comprises a subunit  $\alpha 11$  and a subunit  $\beta$ , or the subunit  $\alpha 11$  thereof, and homologues or fragments of said integrin or subunit  $\alpha 11$ , and a polynucleotide and a oligonucleotide coding for said integrin subunit  $\alpha 11$ .

- A method of gene therapy, whereby a vector comprising a polynucleotide or oligonucleotide coding for a subunit  $\alpha 11$  of an integrin heterodimer, or for homologues or fragments thereof, which polynucleotide or oligonucleotide comprises essentially the nucleotide sequence shown in SEQ ID NO: 1 or parts thereof, and optionally a second vector comprising a polynucleotide or oligonucleotide coding for a subunit  $\beta$  of said integrin heterodimer, is administered to a subject suffering from pathological conditions involving said subunit  $\alpha 11$ .

- A method of using binding entities having the capability of binding specifically to binding sites of a integrin subunit  $\alpha 11$  comprising substantially the amino acid sequence shown in SEQ ID No. 1, or of an integrin heterodimer comprising said subunit  $\alpha 11$  and a subunit  $\beta$ ,

or to homologues or fragments thereof, for promoting adhesion of cells.

- A method of using an integrin heterodimer comprising an integrin subunit  $\alpha 11$  and a subunit  $\beta$ , or the subunit  $\alpha 11$  thereof, or homologues or fragments of said integrin or subunit  $\alpha 11$ , as a target for anti-adhesive drugs or molecules in tissues where adhesion impairs the function of the tissue.

- A method of in vitro detecting the presence of integrin binding entities, comprising interaction of an integrin heterodimer comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , or the subunit  $\alpha 11$  thereof, or homologues or fragments of said integrin or subunit, with a sample, thereby causing said integrin, subunit  $\alpha 11$ , or homologue or fragment thereof, to modulate the binding to its natural ligand or other integrin binding proteins present in said sample.

- A method of in vitro studying consequences of the interaction of a human heterodimer integrin comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , or the subunit  $\alpha 11$  thereof, or homologues or fragments of said integrin or subunit, with an integrin binding entity and thereby initiate a cellular reaction. In one embodiment of this method, the consequences of said interactions are measured as alterations in cellular functions.

- A method of using a polynucleotide or oligonucleotide encoding an integrin subunit  $\alpha 11$  or homologues or fragments thereof as a target molecule.

One embodiment of this method comprises hybridising a polynucleotide or oligonucleotide to the DNA or RNA encoding the integrin subunit  $\alpha 11$  or homologue or fragment thereof, which polynucleotide or oligonucleotide fails to hybridise to a polynucleotide or oligonucleotide encoding an integrin subunit  $\alpha 10$ .

- A method of using binding entities having the capability of binding specifically to an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID

No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having similar biological activity, for promoting adhesion of chondrocytes and/or osteoblasts to surfaces of implants to stimulate osseointegration.

- A method of using an integrin heterodimer comprising an integrin subunit  $\alpha 11$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or homologues or fragments of said integrin or subunit  $\alpha 10$ , as a target for anti-adhesive drugs or molecules in tendon, ligament, skeletal muscle or other tissues where adhesion impairs the function of the tissue.

- A method of stimulating, inhibiting or blocking the formation of cartilage or bone, comprising administration to a subject a suitable amount of a pharmaceutical agent or an antibody which is capable of using an integrin heterodimer comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , or the subunit  $\alpha 11$  thereof, or homologues or fragments of said integrin or subunit  $\alpha 11$ , as a target molecule.

#### EXPERIMENTAL PROCEDURES

##### Cell cultures

The human fetal myoblast/myotube cultures were derived from clone G6 originating from a thigh muscle of a 73-day old aborted fetus ((39); referred to as G6 hereafter). Cultures of G6 and 2.5 years postnatal human satellite cells XXVI, a gift from Dr. Helen Blau (Stanford University, CA), were grown as reported earlier (39). Human rhabdomyosarcoma cell lines RD (ATCC No. CCL-136) and A204 (ATCC No. CRL-7900) were grown in DMEM (Swedish Agricultural University, Uppsala) supplemented with 10% fetal calf serum.

##### RNA isolation and cDNA synthesis

Total RNA from G6 and XXVI myoblasts, the same cells differentiated for 3 or 7 days, and RD and A204 cell lines, was isolated using the RNeasy Midi kit (Qiagen)

according to the manufacturer's instructions. Poly-A RNA was extracted from total RNA of G6 and XXVI cells using Dynabeads mRNA DIRECT kit (DYNAL A.S., Norway).

PCR based cloning and generation of human  $\alpha$ 11 probes

5 First strand cDNA was generated from 1  $\mu$ g of G6 mRNA using a reverse transcription PCR-kit (Perkin-Elmer). Advantage cDNA Polymerase Mix (Clontech) was used in PCR amplifications using two different pairs of primers:

10 (1) 5' ACG GGA GAC GTG TAC AAG TG 3' (forward), 5'-AAA GTG CTG AAC CTC CAC CC-3' (reverse) and (2) 5'-CAC CAT CCA CCA GGC TAT GC -3' (forward), 5'-TTA GCG TTC CGT TAT AAA CA -3' (reverse). The PCR conditions were: 94°C, 4 min. ("hot start"); 94°C, 30 s; 55°C, 30 s; and 72°C, 1 min., for 25 cycles. Two products, named PCR1 and PCR2,

15 were obtained (figure 1), subcloned into the plasmid vector TA (Invitrogen), and sequenced. A single product of 1,4 kb in size, named PCR 3 (figure 1), was amplified using primers 1 (forward) and 2 (reverse), and human heart Marathon-Ready cDNA (Clontech) as template. Anneal-

20 ing temperatures in the applied touch-down program were: 68°C, 1 min., 5 cycles; 65°C, 1 min., 5 cycles; 60°C, 1 min., 25 cycles. Other steps were as described above. After the final cycle the reactions were extended for additional 7 min. at 72°C followed by a hold step at 4°C.

25 To obtain a sequence covering the 5' end, Rapid Amplification of cDNA Ends (RACE) was employed according to the manufacturer's instructions (Marathon cDNA Amplification kit, Clontech) using cDNA prepared from G6 mRNA and the gene specific antisense primer: 5'-CTT GGA GAA CCT GAA

30 GTT GGA GTT GAC -3'. Amplification was carried out applying the "touch-down" program (see above). To identify relevant products, 10  $\mu$ l of each RACE product was resolved on 1% agarose gel and subjected to Southern blot analysis as described previously (40). PCR2 (see above)

35 was labeled with [ $\alpha$ -<sup>32</sup>P]dCTP (Amersham Pharmacia Biotech, Sweden) using the RedyPrimeII DNA labeling system (Amersham Pharmacia Biotech, UK), and used as a hybridi-



zation probe. One specific signal was detected. Corresponding cDNA was purified (Gel Extraction kit, Quagen), cloned into the TA vector and sequenced (see figure 1).

#### Screening of cDNA libraries

5 A  $\lambda$ ZAP custom made G6 cDNA library (Stratagene, USA) was screened with PCR2 (see above) as a probe. The screening procedure (carried out as described in (40)) resulted in two clones representing the 5' non-coding region and the beginning of the coding part of integrin  $\alpha 11$  (figure  
10 1). To obtain an additional sequence, a human uterus 5'-stretch  $\lambda$ gt11 cDNA library (Clontech) was screened with a mixture of PCR1 and PCR2 as probes. The probes were labeled with [ $\alpha$ - $^{32}$ P]dCTP using the Ready-To-Go DNA labeling beads (Amersham Pharmacia Biotech, Sweden). Three clones  
15 (1.1-1.3 in figure 1) representing parts of  $\alpha 11$  cDNA, were obtained. Rescreening of the human uterus 5'-stretch  $\lambda$ gt11 cDNA library with the probe  $\lambda$ 290 (corresponding to 2183-2473 in Fig. 1) yielded three more clones (2.1-2.3, figure  
20 1) covering the rest of  $\alpha 11$  cDNA. Positive clones were plaque purified, the phage DNA isolated using the Lambda Midi kit (Qiagen) and then sub-cloned into the Bluescript SK or pUC19 plasmid vectors before sequencing.

#### Northern hybridization

A filter containing 6  $\mu$ g of the poly-A RNA from G6  
25 and XXVI cells and 10  $\mu$ g of the total RNA from RD and A204 cell lines, and a Human Multiple Tissue Northern Blot containing poly-A RNA from adult human tissues (Clontech), were hybridized at 68°C in ExpressHyb solution (Clontech) with probes labeled as described above.  
30 The probes used were PCR1, PCR2, cDNA clone 1.3 (figure 1), 3RA (1.8 kb cDNA specific for human integrin  $\alpha 1$  mRNA, a generous gift from E.E. Marcantonio (Columbia University, New York), a 1.1 kb cDNA clone recognizing human G3PHD mRNA and a 1.8 kb cDNA clone recognizing human  $\beta$ -  
35 actin (both from Clontech).

cDNA sequencing and sequence analysis

All PCR fragments and cDNA clones were sequenced on both strands either manually (29) or using ABI 310 Genetic Analyzer automatic sequencer. Sequences were analyzed with the aid of MacVector™ 6.0, DNA Star, Faktura™NEW 1.2.0, and Sequence Navigator 1.0.1 software programs. A distance tree of all I-domain containing integrin  $\alpha$  subunits was assembled using SEAVIEW and PHYLO-WIN softwares (41). Percent similarity between every two members in the I-domain integrin subfamily was calculated by a formula  $I=(1-D) \times 100$ , where "I" is identity and "D" is distance.

Antibodies

A polyclonal antiserum ( $\alpha$ 11 cyt) was produced against the peptide CRREPGLDPTPKVLE from the integrin  $\alpha$ 11 cytoplasmic domain. Peptide synthesis and conjugation to Keyhole limpet hemocyanin, immunization of rabbits and affinity purification was performed at Innovagen AB (Lund, Sweden). The monoclonal antibody Mab 13 against integrin  $\beta$ 1 was obtained from S.K. Akiyama (NIEHS, NIH). Monoclonal antibodies to integrin  $\alpha$ 1 (clone FB12, sold as MAB 1973) and integrin  $\alpha$ 2 (clone BHA2.1 sold as Mab 1998) were both obtained from Chemicon, Temecula, CA. The monoclonal antibody to vinculin (clone hVIN-1) was from Sigma (Saint Louis, MO, USA). Secondary fluorescent antibodies (CY3™-coupled goat-anti rabbit IgG and FITC-coupled goat anti-mouse IgG of multiple labeling grade) were from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA, USA).

Immunoprecipitation and SDS-PAGE

G6 and XXVI cells were labeled with [ $^{35}$ S] cysteine/methionine and subjected to immunoprecipitation and SDS-PAGE as reported previously (38). The two-step procedure used to dissociate integrin heterodimers was carried out as follows. After incubation of samples with  $\beta$ 1 antibody and capture with GammaBind G Sepharose (Amersham Pharmacia Biotech, Uppsala, Sweden), 100  $\mu$ l of 1% SDS was

added to the washed beads which were then boiled for 5 minutes. 10 mM Tris-HCl, pH 7.4, 0.15 M NaCl and 1% Triton X-100 was added to a final volume of 1ml and the lysate was incubated with GammaBind 3 Sepharose for 1 hour. The incubation with GammaBind G was performed in order to ensure that no reactive  $\beta 1$  antibodies remained. After removal of GammaBind G Sepharose,  $\alpha 11$  integrin antibody was added for additional 2 hours, followed by capture with protein A Sepharose (Amersham Pharmacia Biotech) and boiling in SDS-PAGE sample buffer.

#### Chromosomal localization

Chromosomal localization of the human integrin  $\alpha 11$  was performed by using a combination of FISH (Fluorescent In Situ Hybridization) technique and DAPI (4',6-diamidino-2-phenylindole) banding essentially as described earlier (42). As a hybridization probe, the 1.4 kb RT-PCR product PCR3 was used.

#### Surface iodination and affinity chromatography

Cultured XXVI cells were surface iodinated as described (38). Labeled cells were solubilized in 1 ml of solubilization buffer (10 mM Tris-HCl pH 7.4, 15 mM NaCl, 1% Triton X-100, 1mM  $MgCl_2$ , 1 mM  $CaCl_2$ , 1mM  $MnCl_2$ ), centrifuged at 14000 g for 20 min., and soluble membrane proteins were applied to a collagen type I Sepharose (bovine collagen type I from Vitrogen (Collagen Corp., Palo Alto) coupled to CNBr-activated Sepharose CL-4B at 3 mg/ml gel as described (14)), equilibrated in solubilization buffer. Following a one hour incubation the column was washed extensively with buffer A (10 mM Tris-HCl pH 7.4, 50 mM NaCl, 1 mM  $MnCl_2$ , 0.1% Triton X-100) and by 10 column volumes of buffer A without NaCl. Bound proteins were eluted with 20 mM EDTA, 10 mM Tris-HCl pH 7.4, 0.1% Triton X-100. Peak fractions were pooled and concentrated by immunoprecipitation with  $\beta 1$  integrin and  $\alpha 11$  integrin antibodies as described under Immunoprecipitation and SDS-PAGE. Eluted fractions and captured

proteins were analyzed on 7.5% SDS-PAGE gels followed by autoradiography.

#### Indirect immunofluorescence

Cells cultured on coverslips were washed in serum-free medium and fixed for 6 min. in acetone at -20°C. Non-specific binding sites were blocked by incubating with 10% goat serum diluted in phosphate buffered saline. In the double immunofluorescence staining protocol, primary antibodies (anti- $\alpha$ 11 cyt (rabbit antibody) and anti-vinculin (mouse antibody)) were simultaneously incubated with fixed cells for 1.5 hours at +37°C. Specifically bound antibodies were detected using anti-rabbit Cy3 IgG and anti-mouse FITC IgG. Stained cells were mounted in Vectashield™ mounting medium (Vector Laboratories, Inc., Burlingame, CA, USA) and visualized and photographed under a Zeiss light microscope equipped with optics for observing fluorescence.

#### RESULTS AND DISCUSSION

##### 20 cDNA cloning of a novel integrin $\alpha$ -chain

In order to determine the nature of the integrin chain that we had previously characterized on human fetal muscle cells and named  $\alpha$ mt (38), a number of approaches were used. Applying PCR with mRNA from fetal muscle cells as template together with degenerate primers to conserved regions of integrin  $\alpha$  subunits (43) we amplified cDNA for  $\alpha$ 1,  $\alpha$ 4,  $\alpha$ 5,  $\alpha$ 6 and  $\alpha$ v integrin chains (data not shown), but failed to amplify the novel integrin. However, while searching through the literature we came across two integrin sequences obtained in a subtractive hybridization protocol comparing human primary myoblasts with the rhabdomyosarcoma cell line RD (44). After having confirmed that these sequences could be amplified by PCR from human fetal G6 myoblast cDNA, PCR was performed assuming that these sequences were derived from the same transcript. In this manner a 1.4 kb cDNA fragment with integrin-like sequence was obtained. Screening of a human fetal myo-

blast cDNA library and 5' RACE yielded additional 5' sequence. We determined the mRNA expression pattern in a number of human tissues (see below) and observed a high mRNA expression in the uterus. Screening of a uterus cDNA library resulted in the identification of the complete open reading frame. A schematic illustration of the cloning strategy is shown in figure 1.

cDNA sequence and predicted amino acid sequence of  $\alpha 11$  integrin chain

By sequence analysis of cDNA clones and 5' RACE products we obtained a continuous sequence of 3983 nucleotides (nt) composed of 90 nt 5' non-coding sequence, 3564 nt open reading frame, and 326 nt 3' non-coding sequence. Translation of the sequence predicts an integrin  $\alpha$ -chain like precursor of 1188 amino acids including a 22 amino acid long signal peptide (fig. 2, GenBank accession No. AF137378). The mature 1166 amino acid long peptide is larger than any other currently identified integrin  $\alpha$ -chain (the closest being  $\alpha E$ , composed of 1160 amino acids (45)). The 1115 amino acid long predicted extracellular domain contains 7 FG-GAP repeats in the amino-terminal end with an inserted I-domain between repeats 2 and 3. The I-domain consists of 195 amino acids and includes a conserved MIDAS motif. In addition to the metal chelating site in the I-domain, three additional potential divalent cation binding motifs with the consensus sequence DXD/NXDXXXD are present in repeats 5-7. A total of 20 cysteines are located in the extracellular domain. Of these, 16 are conserved in the most closely related integrin  $\alpha 10$  and  $\alpha 1$  chains and they may contribute to intramolecular disulphide bonds. The two non-conserved cysteines found at positions Cys 606 and Cys 988 most likely represent free unpaired cysteines while the two non-conserved cysteines Cys 806 and Cys 817 may pair to form a disulphide bond. Mapping of the cysteines in the suggested  $\beta$ -propeller structure shows that the first three disulphide bonds are likely to stabilize

blades one and two of the  $\beta$ -propeller whereas the remaining bonds are found outside the propeller region, in the stalk region towards the transmembrane domain. 16 potential N-glycosylation sites are present in  $\alpha 11$ . A search for sequence motifs reveals the presence of a 22 amino acid leucine zipper motif starting at position 951, and a 17 amino acid sequence starting at position 1082, which is similar to sequences found in G-protein coupled receptors. These sequences might represent functional domains of importance for protein-protein interactions.

The transmembrane region (amino acids 1142-1164) is 23 amino acids long and is followed by a cytoplasmic tail of 24 amino acids. The cytoplasmic tail contains the sequence GFFRS instead of the conserved GFFKR sequence, found in all other  $\alpha$ -chains except  $\alpha 8$ - $\alpha 10$ . It will be interesting to determine the importance of this sequence in defining the cytoplasmic domain as well as its possible ability to bind calreticulin and other intracellular components.

#### Comparison of integrin $\alpha 11$ chain with other integrin $\alpha$ chains

Alignment of the predicted  $\alpha 11$  integrin amino acid sequence with other integrin sequences shows the highest overall identity with  $\alpha 10$  (42% identity),  $\alpha 1$  (37% identity), and  $\alpha 2$  (35% identity), followed by the remaining I-domain containing integrin subunits. Of the non I-domain containing integrins,  $\alpha 4$  and  $\alpha 9$  are the most similar to  $\alpha 11$ . A distance tree shows that  $\alpha 10$  and  $\alpha 11$  form a separate branch from the most closely related  $\alpha 1$  and  $\alpha 2$  integrin chains (fig. 3). The similarity with other integrins is particularly high in the N-terminal  $\beta$ -propeller part but lower in the stalk region. Comparison of  $\alpha 1$  integrin with  $\alpha 2$  integrin has pointed to the presence of a 36-residue insert in the  $\beta$ -propeller region of  $\alpha 1$  integrin chain (15). Like  $\alpha 1$  chain,  $\alpha 11$  also contains inserted amino acids not present in the other I-domain containing integrin chains. however, in the  $\alpha 11$

chain these are found within the stalk region at amino acids 804-826. The exact border of the predicted insertion varies depending on the alignment method and the parameters chosen, but is predicted to span at least 22 amino acids. The insert shows no significant similarity to other integrin sequences and contains two cysteines likely to form a disulphide bond (see fig. 2). We do not believe that the predicted inserted sequence represents a cloning artifact since it is present in three independently analysed clones. Other examples of non I-domain inserted sequences are found in the *Drosophila*  $\alpha$ PS2 chain, where developmentally regulated splicing in the ligand binding region modulates ligand affinity (46). In  $\alpha 7$  integrin chain, splicing in the extracellular domain between predicted blades 2 and 3 in the  $\beta$ -propeller generates X1 and X2 variants, affecting the binding to laminin-1 in a cell-specific manner (47). In the more closely related  $\alpha 1$  integrin chain the 38 extra amino acids are present in a position that is predicted to be in the beginning of the sixth blade of the 7-bladed propeller. So far there is no evidence that the extra amino acids in either  $\alpha 1$  or  $\alpha 11$  arise by alternative splicing. In  $\alpha 11$  the predicted inserted region is outside the  $\beta$ -propeller and most likely does not directly affect ligand binding, but might instead be involved in modifying protein-protein interactions or be important for outside-in or inside-out signalling. In this regard it is interesting to note that tetraspan proteins by binding to the stalk region of certain integrin  $\alpha$ -chains can recruit PI-4 kinase and protein kinase C to integrin complexes (48). Likewise the extracellular membrane-proximal parts of certain integrin  $\alpha$ -chains have been shown to be involved in Shc-mediated integrin signalling (49).

Analysis of sequences identified during screening for genes upregulated during tadpole regression revealed a partial sequence, which at the time was reported to show the highest similarity to integrin  $\alpha 1$  (41% identity;

(50). This sequence, when translated (amino acids 1-116), shows 71% identity to human  $\alpha 11$  and thus most likely represents the *Xenopus* orthologue of  $\alpha 11$  rather than that of the  $\alpha 1$ . These data suggest that  $\alpha 11$  is well conserved during evolution.

#### Chromosomal localization of the integrin $\alpha 11$ gene

A fluorescent cDNA probe was used for in situ hybridization on metaphase chromosome spreads. The analysis shows that the integrin  $\alpha 11$  gene (ITGA11) is located on chromosome 15q23 (fig. 4). The genes for I-domain containing integrins  $\alpha 1$  and  $\alpha 2$  are both present on chromosome 5 (51,52), just as the genes for the closely related  $\beta 2$  integrin associated  $\alpha$ -chains all map to chromosome 16 (53). Interestingly, the  $\alpha 11$  gene and the closely related  $\alpha 1$  and  $\alpha 2$  genes, map to different chromosomes. It will be of evolutionary interest to determine the chromosomal localization of the integrin  $\alpha 10$  gene. Curiously, a form of Bardet-Biedl syndrome characterized by retinitis pigmentosa, polydactyly, obesity, hypogenitalism, mental retardation, and renal anomalies maps to 15q22-23 (54). Future studies will clarify a possible linkage of ITGA11 to Bardet-Biedl syndrome.

#### Expression pattern of $\alpha 11$ mRNA in adult tissues

Northern blot analysis of mRNA from various adult human tissues shows the highest level of expression of  $\alpha 11$  in adult human uterus. A strong signal is also noted in heart, while intermediate levels of  $\alpha 11$  mRNA are present in skeletal muscle and intermediate to low levels in other adult tissues tested (fig. 5 and data not shown). For a comparison, the same blot was probed for the closely related  $\alpha 1$  integrin mRNA (fig. 5). A striking difference in the expression levels of  $\alpha 1$  and  $\alpha 11$  was observed in the smooth muscle rich uterus, which appears to lack  $\alpha 1$ . Immunohistochemical analysis and in situ hybridizations will elucidate the detailed distribution of  $\alpha 11$  protein and mRNA in muscle and other tissues. Neither  $\alpha 1$  (33) nor  $\alpha 2$  (55) are present in muscle fibers, and the



distribution of  $\alpha 10$  in skeletal muscle tissues is not known (5). Hence, no I-domain containing integrin has so far been reported to be expressed in the skeletal muscle sarcolemma. Recently the gene for  $\alpha 1$  integrin was inactivated in mice, resulting in mice with an apparently normal phenotype (56). More careful analysis revealed a phenotype characterized by a hypocellular skin (57) and aberrant regulation of collagen synthesis (58). It will be interesting to compare sites of overlapping expression between  $\alpha 1$ ,  $\alpha 2$  and  $\alpha 10$  integrins, and use reagents to  $\alpha 10$  and  $\alpha 11$  to examine possible functional compensatory mechanisms in  $\alpha 1$  integrin-deficient mice.

#### Biochemical characterization of $\alpha 11$ protein

Following the cloning of the full-length  $\alpha 11$  integrin cDNA it was essential to determine if the predicted amino acid sequence was identical to the novel uncleaved  $\beta 1$  integrin-associated  $\alpha$ -chain that we had previously noted to be upregulated during in vitro differentiation of human myoblasts (38). To answer this question we raised antibodies to the cytoplasmic tail of the integrin  $\alpha 11$  chain. Immunoprecipitation from the human satellite cells showed that the antibodies precipitated a 145 kDa  $\alpha 11$  band associated with a 115 kDa  $\beta 1$  band (fig. 6, panel A) in SDS-PAGE under non-reducing conditions. Under reducing conditions the  $\alpha 11$  band migrated as 155 kDa (see fig. 6, panel B). From the translated amino acid sequence an Mr of 133 400 is predicted for the  $\alpha 11$  chain. Taking the 16 potential glycosylation sites into account this fits well with the observed 155 kDa band in SDS-PAGE. Under non-reducing conditions the 145 kDa band is distinctly larger than  $\alpha 2$  (fig. 6, panel A) and  $\alpha 10$  integrin chains which co-migrate as 140 kDa bands and  $\alpha 11$  migrates well below the 180 kDa integrin  $\alpha 1$  band. The  $\alpha 2$  (59) and  $\alpha 10$  (5) chains both contain 10 potential glycosylation sites whereas  $\alpha 1$  contains 26 glycosylation sites (60). The intermediate size of  $\alpha 11$  in SDS-PAGE compared with  $\alpha 1$

and  $\alpha 2/\alpha 10$  is thus most likely a result of differential glycosylation.

To show that  $\alpha 11$  is associated with the  $\beta 1$  subunit a two-step immunoprecipitation procedure was performed.

5 Integrins were first precipitated with a monoclonal anti- $\beta 1$  integrin antibody and GammaBind G captured integrins were then dissociated by boiling in 1% SDS. In the second step, SDS was diluted tenfold and antibodies to  $\alpha 11$  were added. As shown in fig. 6 panel A antibodies to  $\alpha 11$  immunoprecipitate only the 145 kDa band from the dissociated precipitate initially captured with  $\beta 1$  antibodies.

Induction of  $\alpha 11$  mRNA and protein during myogenic differentiation in vitro

15 It has previously been determined that  $\alpha mt$  is the major integrin  $\alpha$ -chain that is up-regulated during myogenic differentiation on human fetal myoblasts in vitro (38). To compare  $\alpha 11$  levels in myoblasts and myotubes, immuno-precipitates were analyzed from myoblast cultures in pro-liferation medium, and from parallel  
20 cultures allowed to differentiate and form myotubes in differentiation medium for 7 days. Immunoprecipitation with both  $\beta 1$  and  $\alpha 11$  antibodies showed that  $\alpha 11$ , like  $\alpha mt$ , is strongly up-regulated at the protein level in differentiation cultures of human fetal muscle cells and  
25 satellite cells (fig. 6, panel B). To determine if the up-regulation occurs at the mRNA or protein level we analyzed  $\alpha 11$  mRNA from different differentiation stages (day 1, day 3 and day 7) (fig. 6, panel C). Already at day 3 in differentiation medium a strong up-regulation of  
30  $\alpha 11$  mRNA was noted, establishing that the up-regulation of  $\alpha 11$  integrin protein occurs as a result of increased transcription or mRNA stability. Based on similar SDS-PAGE migration patterns, similar behavior under reducing conditions, association with  $\beta 1$  integrin chain, and up-  
35 regulation during in vitro differentiation of human fetal myoblasts, the present data show that  $\alpha 11$  integrin is identical with  $\alpha mt$ .

Analysis of mRNA from the two rhabdomyosarcoma cell lines RD and A204 (fig. 6, panel C) did not provide evidence for the presence of  $\alpha 11$  in either cell line. Based on the observed up-regulation of  $\alpha 11\beta 1$  in human fetal muscle cells and the presence of  $\alpha 11$  message in adult muscle we suggest that  $\alpha 11$  integrin might be involved in early steps of muscle formation and that it in adult muscle tissues may fulfill a stabilizing role. The  $\alpha 7$  integrin subunit is a major  $\beta 1$ -associated integrin chain in muscle, but genetic deletion of  $\alpha 7$  leads to a fairly mild muscular dystrophy (30).

Ligand binding specificity of  $\alpha 11\beta 1$  integrin

So far identified I-domain containing integrins of the  $\beta 1$  integrin subfamily all bind collagens (5,15,59). For  $\alpha 1$  and  $\alpha 2$  this binding capacity has been shown to reside within the I-domain (17,18). To determine if  $\alpha 11\beta 1$  also binds collagen we performed collagen type I Sepharose chromatography of membrane proteins from surface-iodinated XXVI satellite cells. Direct analysis of the EDTA eluate revealed weak bands corresponding to the positions of  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 11$  and  $\beta 1$  in parallel immunoprecipitations (fig. 7, panel 1). The EDTA eluate was concentrated by immunoprecipitation with  $\beta 1$  and  $\alpha 11$  antibodies. As shown in figure 7, a prominent  $\alpha 11$  band is present in the collagen I Sepharose eluate. The relatively weak  $\beta 1$  band in the proteins captured with  $\alpha 11$  antibodies indicates that the  $\alpha 11\beta 1$  heterodimer partly dissociates in the presence of EDTA. To visualize the interaction of  $\alpha 11\beta 1$  integrin with collagen I in intact cells, myogenic cells expressing  $\alpha 11\beta 1$  were trypsinized and plated on collagen and fibronectin for 1 hour. The ability to form focal contacts was investigated by double immunofluorescence staining for  $\alpha 11$ -chain and vinculin. As seen in panel 2 of figure 7  $\alpha 11$  localizes to vinculin positive focal contacts on collagen but not on fibronectin. Binding studies with  $\alpha 11$  I-domain expressed as a bacterial GST-fusion protein also confirmed a specific

affinity for collagen I (unpublished M. Höök, R. Rich, R. Owens). Stable transfections of  $\alpha 11$  cDNA into cells with various integrin backgrounds will allow a more detailed study of  $\alpha 11\beta 1$  interactions with different collagen, and possibly also laminin, isoforms. Combined with in vivo distribution studies of  $\alpha 11\beta 1$  this is likely to yield valuable information regarding the in vivo ligands for  $\alpha 11\beta 1$  in different tissues.

$\alpha 11$  integrin protein distribution in human embryo

Morphologically normal human embryos (aged from 4 to 8 post-ovulatory weeks) were obtained from legal abortions induced by Mifepristone (RU486) at Hopital Broussais in Paris. All procedures were approved by the Ethical Committee of Saint-Vincent de Paul Hospital in Paris.

Each sample was first examined macroscopically during dissection under a stereo-microscope. The development stage of the embryos was determined using established criteria. Tissues were collected shortly after delivery and frozen within the first 24 h post mortem on dry ice and stored at  $-80^{\circ}\text{C}$  until used. Seven micron-thick cryostat sections were mounted on slides previously coated with a 2% 3-aminopropyl-triethoxysilane solution in acetone. The cryosection was left unfixed prior to blocking of non-specific binding sites with 10% goat serum diluted in phosphate buffered saline. For immunofluorescence, the section was incubated with  $\alpha 11$  antibodies 1.5 h at  $+37^{\circ}\text{C}$ . Specifically bound antibodies were detected using goat anti-rabbit Cy3 IgG (Jackson Immunoresearch). The stained tissue section was mounted in Vectashield™ mounting medium (Vector Laboratories Inc.) and visualized and photographed under a Zeiss light microscope equipped with optics for observing fluorescence.

The results obtained are shown in figure 8. High levels of  $\alpha 11$  protein were noted around vertebrae (arrows), in intervertebrae disc (asterisks), around ribs

(thin arrows) and around forming cartilage in the  
forelimb (arrowhead).

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FIGURE LEGENDS

Figure 1. Schematic organization of PCR fragments and cDNA clones representing different parts of the full length sequence of integrin  $\alpha 11$  subunit

A. Clones 1.1-1.3 and 2.1-2.3 are from the first and the second round of screening, respectively. Fragment 0.0 represents a 5' RACE product as well as a clone obtained from screening of the G6 library. PCR fragments 1-3 and a SacI fragment of a clone 1.3, 2290, are marked with thick lines. Names and positions of all the clones on a scheme are shown in tabulated form in B.

B. Names of the PCR-amplified fragments and cDNA clones shown in A are in the left column, and their positions in the full length cDNA of integrin  $\alpha 11$  in the right column.

Figure 2. Nucleotide and deduced amino acid sequence of the human integrin  $\alpha 11$  chain

The putative signal peptide is underlined in bold, I-domain is boxed, potential N-linked glycosylation sites are marked with asterisks, cysteines are underlined, potential divalent cation binding motifs are double underlined and the transmembrane domain is underlined with dashes. A 22 amino acid insert is boxed in bold.

Figure 3. A distance tree of the I-domain containing  $\alpha$ -integrin subfamily members

A tree was assembled based using ClustalW multiple alignment - based SEAVIEW and PHYLOWIN softwares. A scale at the bottom shows percent identity.

Figure 4. Chromosome mapping of ITGA11 gene by fluorescent in situ hybridization (FISH)

A. Left panel shows the FISH signals on human chromosome 15; right panel shows the same mitotic figure stained with 4',6-diamino-2-phenylindole to identify human chromosome 15.

B. Diagram of FISH mapping result for the probe PCR3 based on a detailed analyses of 10 different images. Each

dot represents the double FISH signals detected on human chromosome 15.

Figure 5. Expression of integrin  $\alpha 11$  and  $\alpha 1$  subunit mRNAs in adult human tissues

- 5        Integrin  $\alpha 11$  mRNA and integrin  $\alpha 1$  mRNA were analyzed on a membrane with RNA from various adult human tissues where mRNA loading was normalized with respect to  $\beta$ -actin. Probes used for hybridizations are marked on the left. Size of molecular weight standard is marked to the right.
- 10      Note that the  $\beta$ -actin probe reacts with 2 kb  $\beta/\gamma$  actin transcripts and the muscle specific 1.8 kb  $\alpha$ -actin message.

Figure 6. Biochemical characterization of integrin  $\alpha 11$  chain and upregulation of corresponding protein and mRNA in myogenic cells

- 15      A.     $\alpha 11$  associates with  $\beta 1$  integrin chain. Human XXVI and G6 muscle cells were metabolically labeled with [ $^{35}\text{S}$ ] cysteine/methionine and integrins were immunoprecipitated with the indicated antibodies ( $\beta 1$ ,  $\alpha 2$  and  $\alpha 11$ ). Evidence
- 20      for the association of integrin  $\alpha 11$  with the  $\beta 1$  subunit obtained by treating proteins precipitated with anti- $\beta 1$  antibodies with SDS followed by a second precipitation with  $\alpha 11$  antibodies (ant- $\alpha 11$ +SDS). Precipitated proteins were resolved on 7.5% SDS-PAGE gels in the absence of
- 25      reducing agents, followed by fluorography.

B. Induction of integrin  $\alpha 11$  upon myogenic differentiation in vitro.

- 30      G6 muscle cells were metabolically labeled with [ $^{35}\text{S}$ ] cysteine/methionine when growing in proliferation medium (mb-proliferating myoblasts) and after 7 days in differentiation medium (mt-myotubes). Integrins were precipitated with antibodies to  $\beta 1$  and  $\alpha 11$  and the precipitates were resolved on 7.5% SDS-PAGE gels both under non-reducing (UNREDUCED) and reducing (REDUCED) conditions.
- 35      Lanes 1, 3, 5 and 7 are immunoprecipitations with the antibody to integrin  $\beta 1$ , and lanes 2, 4, 6 and 8 with the antibody to integrin  $\alpha 11$ .

C. Upregulation of integrin  $\alpha 11$  mRNA in differentiated myogenic cells.

mRNA was extracted from G6 and XXVI cells growing under proliferating (p) or differentiating (d) conditions for 3 days (d3) or 7 days (d7). Total RNA was isolated from RD and A204 cells. Following separation of RNA on agarose gel and transfer to the membrane, the filter was hybridized with probes to  $\alpha 11$  integrin ( $\alpha 11$ ) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Size of bands in RNA standard (in kb) are marked to the right.

Figure 7. Ligand binding properties of  $\alpha 11\beta 1$  integrin panel 1: Collagen binding integrins on XXVI cells.

XXVI cells were surface iodinated and integrins were analyzed by immunoprecipitation and collagen I Sepharose affinity chromatography. Immunoprecipitation reveals the presence of  $\beta 1$  integrins (lane 1),  $\alpha 1\beta 1$  (lane 2),  $\alpha 11\beta 1$  (lane 3) and  $\alpha 2\beta 1$  (lane 4) at the surface of XXVI cells. EDTA eluted proteins bound to collagen I Sepharose contain weak band in the position of  $\alpha 1$ ,  $\alpha 11$ ,  $\alpha 2$  and  $\beta 1$  integrin chains (lane 5). Immunoprecipitations with  $\beta 1$  integrin antibodies (lane 6) and  $\alpha 11$  integrin antibodies (lane 7) confirm the presence of  $\alpha 11$  and  $\beta 1$  in the EDTA eluate.

panel 2:  $\alpha 11\beta 1$  localizes to focal contacts on collagen.

Indirect immunofluorescent visualization of vinculin (A, B) and  $\alpha 11$  integrin chain (C, D) in human XXVI satellite cells seeded on collagen type I (A and C) and fibronectin (B and D). Note the localization of integrin  $\alpha 11$  chain to focal contacts of cells allowed to attach to collagen and its complete absence on cells seeded on fibronectin. Vinculin is found in focal contacts on both substrates. A and C show the same cell double stained for both antigens. Scale bar is  $20\mu\text{m}$ .

Figure 8.  $\alpha 1$  integrin protein distribution at 8 weeks of gestation.

Composite of immunohistochemical staining of sagittal section of human embryo at 8 weeks of gestation. Note  
5 high levels of  $\alpha 1$  protein around vertebrae (arrows), in intervertebral disc (asterisks), around ribs (thin arrows) and around forming cartilage in the forelimb (arrowhead).

## CLAIMS

1. A recombinant or isolated integrin subunit  $\alpha 11$   
5 comprising essentially the amino acid sequence shown in  
SEQ ID No. 1, and homologues and fragments thereof.
2. A process of producing a recombinant integrin  
subunit  $\alpha 11$  comprising essentially the amino acid  
sequence shown in SEQ ID No. 1, and homologues and  
10 fragments thereof, which process comprises the steps of
  - a) isolating a polynucleotide comprising a nucleotide sequence coding for a integrin subunit  $\alpha 11$ , of for homologues and fragments thereof,
  - b) constructing an expression vector comprising the  
15 isolated polynucleotide,
  - c) transforming a host cell with said expression vector,
  - d) culturing said transformed host cell in a culture medium under conditions suitable for expression of said  
20 integrin subunit  $\alpha 11$ , of said homologues and fragments, in said transformed host cell, and, optionally,
  - e) isolating the integrin subunit  $\alpha 11$ , or homologues and fragments thereof, from said transformed host cell or said culture medium.
- 25 3. A process according to claim 2, step c, said transforming being an *in vitro* or *in situ* process.
4. A process according to claim 2, step c, said transforming being an *in vivo* process.
5. A process of providing an integrin subunit  $\alpha 11$ ,  
30 of homologues or fragments thereof, whereby said subunit is isolated from a cell in which it is naturally present.
6. An isolated polynucleotide or oligonucleotide comprising a nucleotide coding for an integrin subunit  $\alpha 11$ , or for homologues or fragments thereof, which  
35 polynucleotide or oligonucleotide comprises essentially the nucleotide sequence shown in SEQ ID No. 1 or suitable parts thereof.

7. An isolated polynucleotide or oligonucleotide which hybridises to a polynucleotide or oligonucleotide as defined in claim 4, whereby said isolated polynucleotide or oligonucleotide fails to hybridise to a polynucleotide or oligonucleotide encoding an integrin subunit  $\alpha 10$ .

8. A vector comprising a polynucleotide or oligonucleotide as defined in claim 6 or 7.

9. A cell containing the vector as defined in claim 8.

10. A cell, as generated by the process in steps a) to c) of claim 2, in which a polynucleotide or oligonucleotide coding for an integrin subunit  $\alpha 11$ , or for homologues and fragments thereof, said polynucleotide or oligonucleotide comprising essentially the nucleotide sequence shown in SEQ ID No. 1 or parts thereof, has been stably integrated in the cell genome.

11. Binding sites of the amino acid sequence of the integrin subunit  $\alpha 11$ , or of homologues and fragments thereof, as defined in claim 1, said binding sites having the capability of binding specifically to entities chosen from the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, polyclonal and monoclonal antibodies, and fragments thereof.

12. Binding entities having the capability of binding specifically to integrin subunit  $\alpha 11$ , or to homologues or fragments thereof, as defined in claim 1, which entities are chosen from the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, polyclonal and monoclonal antibodies, and fragments thereof.

13. A recombinant or isolated integrin heterodimer comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , in which the subunit  $\alpha 11$  comprises essentially the amino acid sequence shown in SEQ ID No. 1 or homologues or fragments thereof.

14. A recombinant or isolated integrin heterodimer according to claim 11, wherein the subunit  $\beta$  is  $\beta 1$ .

15. A process of producing a recombinant integrin heterodimer comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , in which the subunit  $\alpha 11$  comprises essentially the amino acid sequence shown in SEQ ID No. 1, or homologues or fragments thereof, which process comprises the steps of
- a) isolating one polynucleotide or oligonucleotide comprising a nucleotide sequence coding for said subunit  $\alpha 11$  of said integrin heterodimer, or for said homologues or fragments thereof, and, optionally, another polynucleotide comprising a nucleotide sequence coding for said subunit  $\beta$  of an integrin heterodimer, or for homologues or fragments thereof,
  - b) constructing an expression vector comprising said isolated polynucleotides or oligonucleotides
  - c) transforming a host cell with said expression vector or vectors,
  - d) culturing said transformed host cell in a culture medium under conditions suitable for expression of said integrin heterodimer, or said homologues or fragments thereof, in said transformed host cell, and, optionally,
  - e) isolating said integrin heterodimer, or said homologues or fragments thereof, from said transformed host cell or said culture medium.

16. A process according to claim 15, step c, said transforming being an *in vitro* process.

17. A process according to claim 15, step c, said transforming being an *in vivo* process.

18. A process of providing an integrin heterodimer comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , as defined in claim 13 or 14, or homologues or fragments thereof, whereby said integrin heterodimer is isolated from a cell in which it is naturally present.

19. A cell containing

- i) a first vector, said first vector comprising a polynucleotide or oligonucleotide coding for a subunit



all of an integrin heterodimer, or for homologues or fragments thereof, which polynucleotide or oligonucleotide comprises essentially the nucleotide sequence shown in SEQ ID No. 1 or parts thereof, and

- 5        ii) a second vector, said second vector comprising a polynucleotide or oligonucleotide coding for a subunit  $\beta$  of said integrin heterodimer.

20. Binding sites of an integrin heterodimer as defined in claim 13 or 14, or of homologues or fragments thereof, said binding sites having the capability of binding specifically to entities chosen among the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, polyclonal and monoclonal antibodies, and fragments thereof.

15        21. Binding entities having the capability of binding specifically to an integrin heterodimer as defined in claim 13 or 14, or to homologues or fragments thereof, said binding entities being chosen among the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, polyclonal and monoclonal antibodies, and fragments thereof.

22. A fragment of an integrin subunit  $\alpha 11$ , which integrin subunit  $\alpha 11$  comprises essentially the amino acid sequence shown in SEQ ID NO: 1, said fragment being a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the extracellular extension region.

23. A fragment according to claim 22, said fragment being a peptide from the cytoplasmic domain comprising essentially the amino acid sequence  
KLGFFRSARRRRREPGLDPTPKVLE.

24. A fragment according to claim 22, which is a peptide comprising essentially the amino acid sequence of the extracellular domain, from about amino acid No. 804 to about amino acid no. 826 of SEQ ID No. 1.

25. A fragment according to claim 22, which is a peptide comprising essentially the amino acid sequence of

the I-domain, from about amino acid No. 159 to about amino acid no. 355 of SEQ ID No. 1.

26. A method of producing a fragment of the integrin subunit  $\alpha 11$  as defined in any one of claims 22-25, which  
5 method comprises a sequential addition of amino acids.

27. A polynucleotide or oligonucleotide coding for a fragment of the integrin subunit  $\alpha 11$  as defined in any one of claims 22-25.

28. Binding sites of an integrin subunit  $\alpha 11$   
10 fragment as defined in any one of claims 22-25, said binding sites having the capability of binding specifically to entities chosen from the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, monoclonal and polyclonal  
15 antibodies, and fragments thereof.

29. Binding entities having the capability of binding specifically to an integrin subunit  $\alpha 11$  fragment as defined in any one of claims 22-25, which binding entities are chosen from the group comprising proteins,  
20 peptides, carbohydrates, lipids, natural integrin binding ligands, monoclonal and polyclonal antibodies, and fragments thereof.

30. A process of using an integrin subunit  $\alpha 11$  comprising essentially the amino acid sequence shown in SEQ  
25 ID No.1 or an integrin heterodimer comprising said subunit  $\alpha 11$  and a subunit  $\beta$ , or homologues or fragments thereof, as a marker or target molecule of cells or tissues expressing said integrin subunit  $\alpha 11$ , which cells or tissues are of animal including human origin.

31. A process according to claim 30, which is a  
30 process for determining the differentiation-state of cells during differentiation, development, in pathological conditions, in tissue regeneration, in transplantation, or in therapeutic and physiological  
35 reparation of tissues.

32. A process according to claim 31, which process is used during pathological conditions involving said subunit  $\alpha 11$ .

5 33. A process according to claim 31, which pathological conditions are comprised within the group comprising damage of muscles, muscle dystrophy, fibrosis and wound healing.

10 34. A process according to claim 31, which pathological conditions are comprised within the group comprising damage of cartilage and/or bone, and cartilage and/or bone diseases.

15 35. A process according to claim 31, which pathological conditions are comprised within the group comprising trauma, rheumatoid arthritis, osteoarthritis and osteoporosis.

36. A process according to claim 30, which is a process for detecting the formation of cartilage during embryonic development.

20 37. A process according to claim 30, which is a process for detecting physiological or therapeutic repair of cartilage and/or muscle.

25 38. A process according to claim 30, which is a process for selection and analysis, or for sorting, isolating or purification of chondrocytes and/or muscle cells.

30 39. A process according to claim 30, which is a process for detecting regeneration of cartilage or chondrocytes during transplantation of cartilage or chondrocytes, respectively, or of muscle or muscle cells during transplantation of muscle or muscle cells, respectively.

40. A process according to claim 30, which is a process for studies of differentiation of chondrocytes or muscle cells.

35 41. A process according to any one of claims 30-40, which is an in vitro process.

42. A process according to any one of claims 30-40, which is an *in situ* process.
43. A process according to any one of claims 30-40, which is an *in vivo* process.
- 5 44. A process according to any one of claims 30-43, whereby a fragment of said integrin subunit  $\alpha 11$  is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the extracellular extension region.
- 10 45. A process according to claim 44, whereby said fragment is a peptide comprising essentially the amino acid sequence KLGFFRSARRRREPGLDPTPKVLE from the cytoplasmic domain.
- 15 46. A process according to claim 44, whereby said fragment is a peptide comprising essentially the amino acid sequence of the extracellular domain, from about amino acid No. 804 to about amino acid no. 826 of SEQ ID No. 1.
- 20 47. A process according to claim 44, whereby said fragment is a peptide comprising essentially the amino acid sequence of the I-domain, from about amino acid No. 159 to about amino acid no. 355 of SEQ ID No. 1.
48. A process according to any one of claims 30-47, whereby a subunit  $\beta$  of the integrin heterodimer is  $\beta 1$ .
- 25 49. A process according to claim 30, whereby said cells are chosen from the group comprising fibroblasts, muscle cells, chondrocytes, osteoblasts, mesenchymally derived cells and stem cells.
- 30 50. A process of using binding entities having the capability of binding specifically to binding sites of an integrin subunit  $\alpha 11$  comprising essentially the amino acid sequence shown in SEQ ID No. 1, or an integrin heterodimer comprising said subunit  $\alpha 11$  and a subunit  $\beta$ , or to homologues or fragments thereof, as markers or
- 35 target molecules of cells or tissues expressing said integrin subunit  $\alpha 11$ , which cells or tissues are of animal including human origin.

51. A process according to claim 50, which is a process for detecting the presence of an integrin subunit  $\alpha 11$  comprising essentially the amino acid sequence shown in SEQ ID No. 1, or of an integrin heterodimer comprising  
5 said subunit  $\alpha 11$  and a subunit  $\beta$ , or of homologues or fragments thereof.

52. A process according to claim 50, which is a process for determining the differentiation-state of cells during differentiation, development, in  
10 pathological conditions, in tissue regeneration, in transplantation, or in therapeutic and physiological repair of tissues.

53. A process according to claim 52, which process is used during pathological conditions involving said  
15 subunit  $\alpha 11$ .

54. A process according to claim 52, which pathological conditions are comprised within the group comprising damage of muscles, muscle dystrophy, fibrosis and wound healing.

20 55. A process according to claim 52, which pathological conditions are comprised within the group comprising damage of cartilage and/or bone, and cartilage and/or bone diseases.

25 56. A process according to claim 52, which pathological conditions are comprised within the group comprising trauma, rheumatoid arthritis, osteoarthritis and osteoporosis.

57. A process according to claim 52 which is a process for detecting the formation of cartilage during  
30 embryonic development.

58. A process according to claim 52, which is a process for detecting physiological or therapeutic reparation of cartilage and/or muscle.

35 59. A process according to claim 52, which is a process for selection and analysis, or for sorting, isolating or purification of chondrocytes and/or muscle cells.

60. A process according to claim 52, which is a process for detecting regeneration of cartilage or chondrocytes during transplantation of cartilage or chondrocytes, respectively, or of muscle or muscle cells during transplantation of muscle or muscle cells, respectively.

61. A process according to claim 52, which is a process for studies of differentiation of chondrocytes or muscle cells.

62. A process according to any one of claims 50-61, which is an *in vitro* process.

63. A process according to any one of claims 50-61, which is an *in situ* process.

64. A process according to any one of claims 50-61, which is an *in vivo* process.

65. A process according to any one of claims 50-61, whereby a fragment of said integrin subunit  $\alpha 11$  is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the extracellular extension region.

66. A process according to claim 65, whereby said fragment is a peptide comprising essentially the amino acid sequence KLGFFRSARRRREPGLDPTPKVLE from the cytoplasmic domain.

67. A process according to claim 65, whereby said fragment is a peptide comprising essentially the amino acid sequence of the extracellular domain, from about amino acid No. 804 to about amino acid no. 826 of SEQ ID No. 1.

68. A process according to claim 65, whereby said fragment is a peptide comprising essentially the amino acid sequence of the I-domain, from about amino acid No. 159 to about amino acid no. 355 of SEQ ID No. 1.

69. A process according to any one of claims 50-68, whereby a subunit  $\beta$  of the integrin heterodimer is  $\beta 1$ .

70. A process according to claim 50, whereby said cells are chosen from the group comprising fibroblasts,

muscle cells, chondrocytes, osteoblasts, mesenchymally derived cells and stem cells.

71. A process for detecting the presence of an integrin subunit  $\alpha 11$ , or of homologues or fragments of said  
5 integrin subunit, on cells, whereby a polynucleotide or oligonucleotide chosen from the group comprising a polynucleotide or oligonucleotide having essentially the nucleotide sequence as shown in SEQ ID No. 1, or homologues or fragments thereof, is used as a marker  
10 under hybridisation conditions, wherein said polynucleotide or oligonucleotide fails to hybridise to a polynucleotide or oligonucleotide encoding an integrin subunit  $\alpha 10$ .

72. A process according to claim 71, which is a  
15 process for determining the differentiation-state of cells during differentiation, development, in pathological conditions, in tissue regeneration, in transplantation, or in therapeutic and physiological reparation of tissues.

73. A process according to claim 72, which process  
20 is used during pathological conditions involving said subunit  $\alpha 11$ .

74. A process according to claim 72, which pathological conditions are comprised within the group  
25 comprising damage of muscles, muscle dystrophy, fibrosis and wound healing.

75. A process according to claim 72, which pathological conditions are comprised within the group  
30 comprising damage of cartilage and/or bone, and cartilage and/or bone diseases.

76. A process according to claim 72, which pathological conditions are comprised within the group comprising trauma, rheumatoid arthritis, osteoarthritis and osteoporosis.

77. A process according to claim 72, which is a  
35 process for detecting the formation of cartilage during embryonic development.

78. A process according to claim 72, which is a process for detecting physiological or therapeutic repair of cartilage and/or muscle.

79. A process according to claim 72, which is a process for selection and analysis, or for sorting, isolating or purification of chondrocytes and/or muscle cells.

80. A process according to claim 72, which is a process for detecting regeneration of cartilage or chondrocytes during transplantation of cartilage or chondrocytes, respectively, or of muscle or muscle cells during transplantation of muscle or muscle cells, respectively.

81. A process according to claim 72, which is a process for studies of differentiation of chondrocytes or muscle cells.

82. A process according to any one of claims 71-81, which is an *in vitro* process.

83. A process according to any one of claims 71-81, which is an *in situ* process.

84. A process according to any one of claims 71-81, which is an *in vivo* process.

85. A process according to any one of claims 71-84, whereby said polynucleotide or oligonucleotide is a polynucleotide or oligonucleotide coding for a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the extracellular extension region.

86. A process according to claim 85, whereby said peptide is a peptide comprising essentially the amino acid sequence KLGFFRSARRRREPGLDPTPKVLE from the cytoplasmic domain.

87. A process according to claim 85, whereby said peptide is a peptide comprising essentially the amino acid sequence of the extracellular domain, from about amino acid No. 804 to about amino acid no. 826 of SEQ ID No. 1.



88. A process according to claim 85, whereby said peptide is a peptide comprising essentially the amino acid sequence of the I-domain, from about amino acid No. 159 to about amino acid no. 355 of SEQ ID No. 1.

5 89. A process according to any one of claims 71-88, whereby a subunit  $\beta$  of the integrin heterodimer is  $\beta 1$ .

90. A process according to claim 71, whereby said cells are chosen from the group comprising fibroblasts, muscle cells, chondrocytes, osteoblasts, mesenchymally  
10 derived cells and stem cells.

91. A pharmaceutical composition comprising as an active ingredient a pharmaceutical agent or an antibody which is capable of using an integrin heterodimer comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , or the subunit  $\alpha 11$   
15 thereof, or homologues or fragment of said integrin or subunit  $\alpha 11$ , as a target molecule.

92. A pharmaceutical composition comprising as an active ingredient a pharmaceutical agent or an antibody which is capable of stimulating cell surface expression  
20 or activation of an integrin heterodimer comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , or the subunit  $\alpha 11$  thereof, or homologues or fragments of said integrin or subunit  $\alpha 11$ .

93. A pharmaceutical composition according to claim  
25 92, for use in stimulating, inhibiting or blocking the formation of muscles, cartilage, bone or blood vessels.

94. A vaccine comprising as an active ingredient at least one member of the group comprising an integrin heterodimer, which heterodimer comprises a subunit  $\alpha 11$   
30 and a subunit  $\beta$ , or the subunit  $\alpha 11$  thereof, and mologues or fragments of said integrin or subunit  $\alpha 11$ , and a polynucleotide and a oligonucleotide coding for said integrin subunit  $\alpha 11$ .

95. A method of gene therapy, whereby a vector  
35 comprising a polynucleotide or oligonucleotide coding for a subunit  $\alpha 11$  of an integrin heterodimer, or for homologues or fragments thereof, which polynucleotide or

oligonucleotide comprises essentially the nucleotide sequence shown in SEQ ID NO: 1 or parts thereof, and optionally a second vector comprising a polynucleotide or oligonucleotide coding for a subunit  $\beta$  of said integrin heterodimer, is administered to a subject suffering from pathological conditions involving said subunit  $\alpha 11$ .

96. A method of using binding entities having the capability of binding specifically to binding sites of an integrin subunit  $\alpha 11$  comprising substantially the amino acid sequence shown in SEQ ID No. 1, or of an integrin heterodimer comprising said subunit  $\alpha 11$  and a subunit  $\beta$ , or to homologues or fragments thereof, for promoting adhesion of cells.

97. A method of using an integrin heterodimer comprising an integrin subunit  $\alpha 11$  and a subunit  $\beta$ , or the subunit  $\alpha 11$  thereof, or homologues or fragments of said integrin or subunit  $\alpha 11$ , as a target for anti-adhesive drugs or molecules in tissues where adhesion impairs the function of the tissue.

98. A method of in vitro detecting the presence of integrin binding entities, comprising interaction of an integrin heterodimer comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , or the subunit  $\alpha 11$  thereof, or homologues or fragments of said integrin or subunit, with a sample, thereby causing said integrin, subunit  $\alpha 11$ , or homologue or fragment thereof, to modulate the binding to its natural ligand or other integrin binding proteins present in said sample.

99. A method of in vitro studying consequences of the interaction of a human heterodimer integrin comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , or the subunit  $\alpha 11$  thereof, or homologues or fragments of said integrin or subunit, with an integrin binding entity and thereby initiate a cellular reaction.

100. A method according to claim 99, whereby the consequences of said interactions are measured as alterations in cellular functions.

50

101. A method of using a polynucleotide or oligonucleotide encoding an integrin subunit  $\alpha 11$  or homologues or fragments thereof as a target molecule.

102. A method according to claim 101, comprising  
5 hybridising a polynucleotide or oligonucleotide to the DNA or RNA encoding the integrin subunit  $\alpha 11$  or homologue or fragment thereof, which polynucleotide or oligonucleotide fails to hybridise to a polynucleotide or oligonucleotide encoding an integrin subunit  $\alpha 10$ .

10 103. A method of using binding entities having the capability of binding specifically to an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having similar biological  
15 activity, for promoting adhesion of chondrocytes and/or osteoblasts to surfaces of implants to stimulate osseointegration.

104. A method of using an integrin heterodimer  
20 comprising an integrin subunit  $\alpha 11$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or homologues or fragments of said integrin or subunit  $\alpha 10$ , as a target for anti-adhesive drugs or molecules in tendon, ligament, skeletal muscle or other tissues where adhesion impairs the  
25 function of the tissue.

105. A method of stimulating, inhibiting or blocking the formation of cartilage or bone, comprising administration to a subject a suitable amount of a pharmaceutical agent or an antibody which is capable of using an  
30 integrin heterodimer comprising a subunit  $\alpha 11$  and a subunit  $\beta$ , or the subunit  $\alpha 11$  thereof, or homologues or fragments of said integrin or subunit  $\alpha 11$ , as a target molecule.

1/11

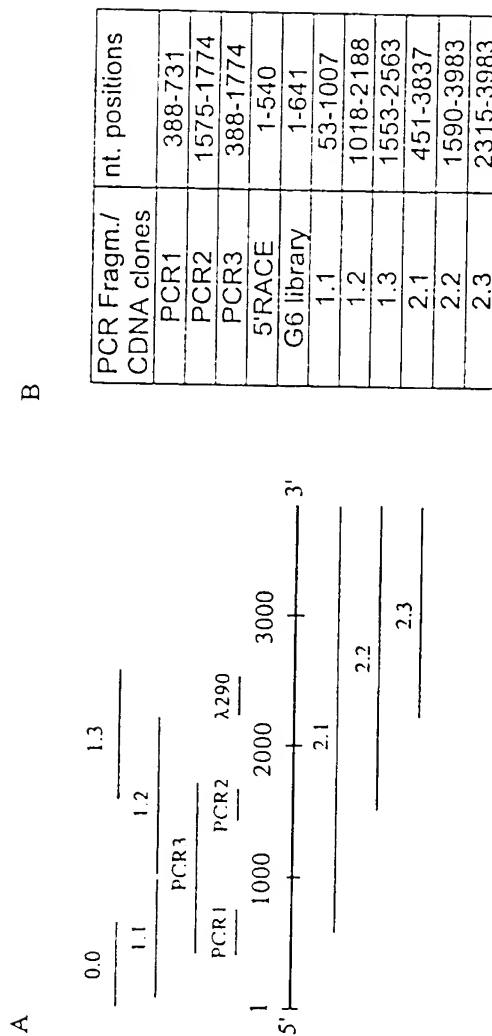


Fig. 1

[illegible]

Fig 2b

TTTTCTCGCAGCTGGTGGGTTCTGCTGGAGCGTTCGTGCTATGCTGGATGGAGCTGTCTAAGGAGACGAGTGCCGGGAAGGTCAATCTCTCCGCGAGTCTTAC 1320  
 F S S H V V E D G V L L G A V G A Y D W N G A V L K E T S A G K V I P L R E S Y 410  
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Fig 2c

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 Q V C N<sup>o</sup> V S Y P F F R A K A K V A F R L D S E F S K S I F L H H L E I E L A A G 930  
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 K L N<sup>o</sup> S S L E R Y D G I G P P F S C I F R I Q N L G L F P I H G M M K I T I P 1010  
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511

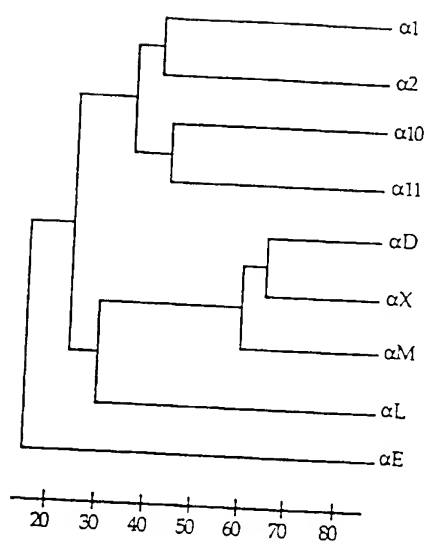
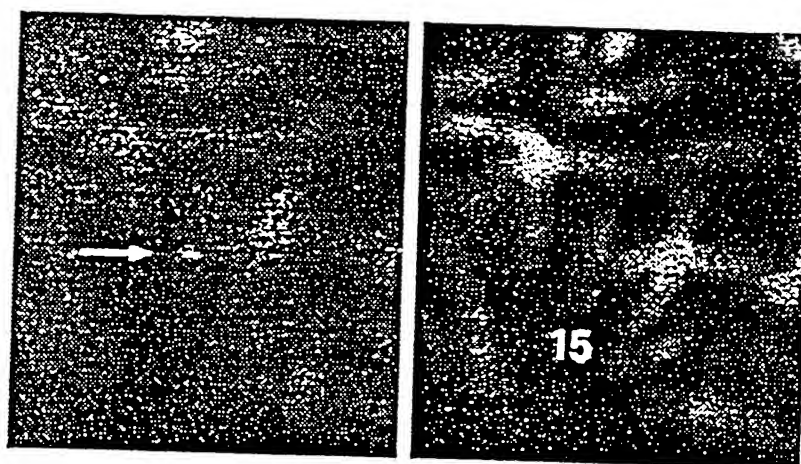


Fig. 3



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A



B

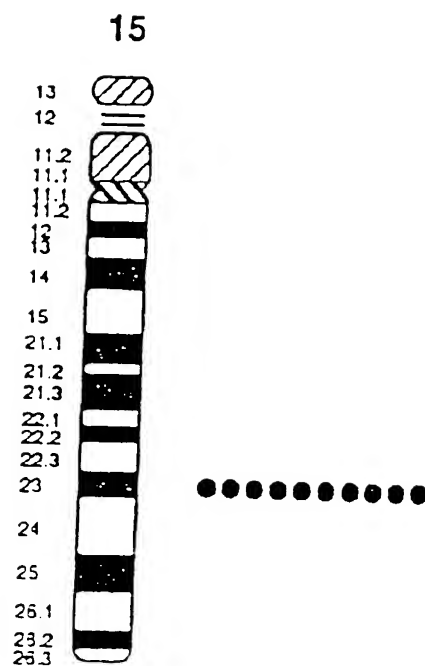


FIG. 4

711

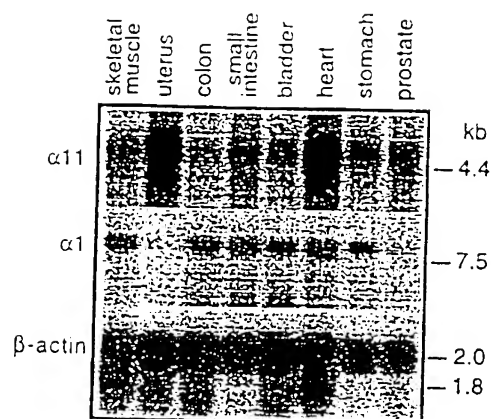


Fig. 5

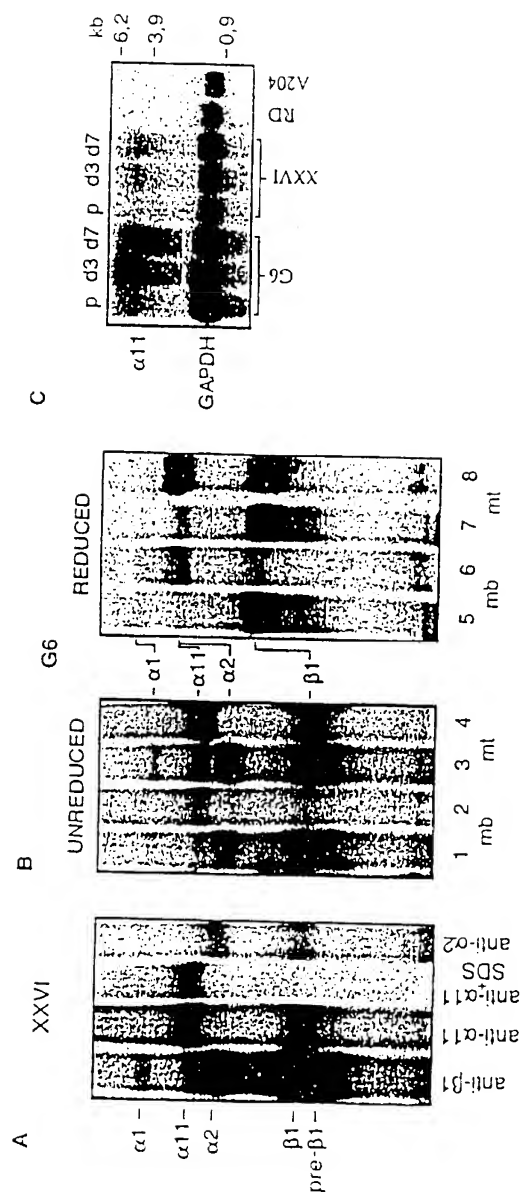


Fig. 6

## Immunoprecipitation    Collagen I Sepharose

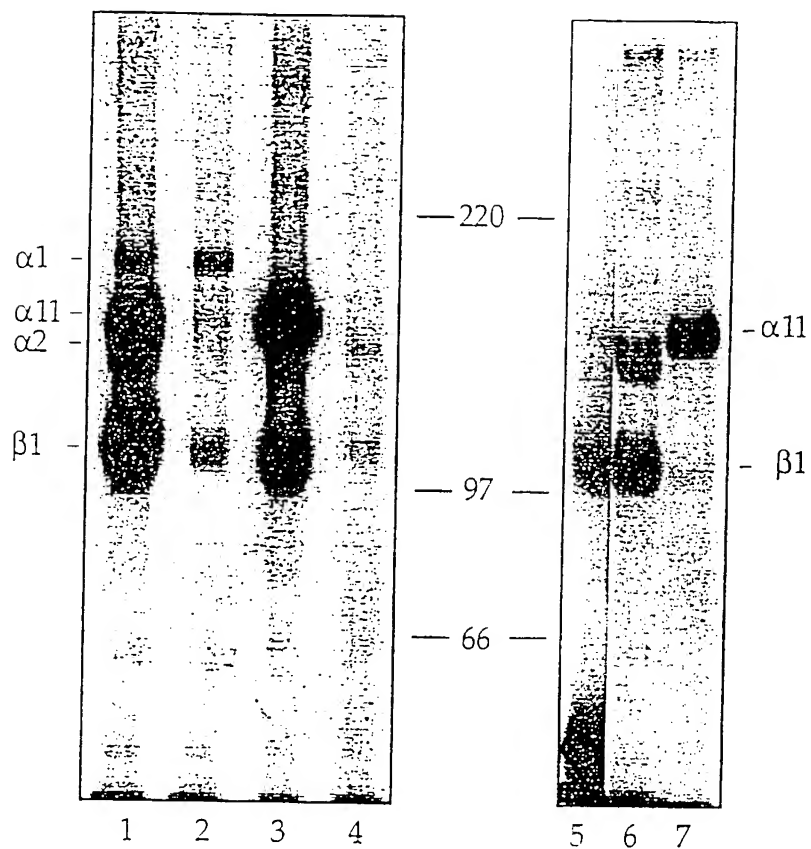


Fig. 7 panel 1

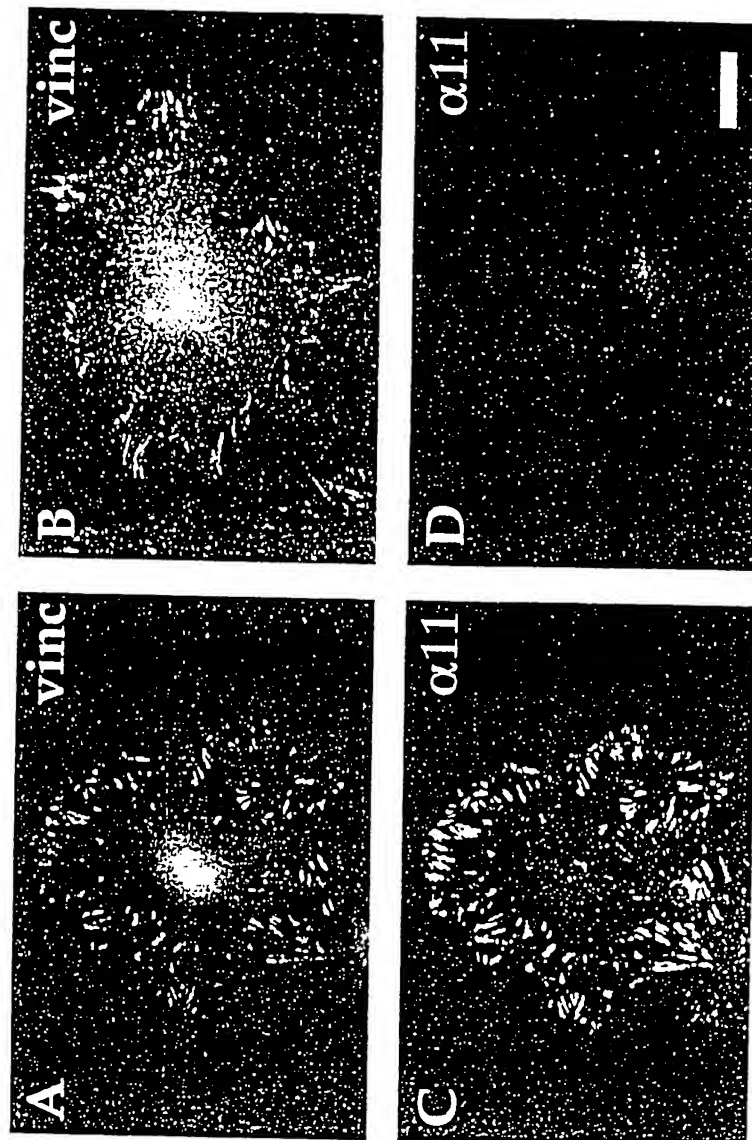


Fig. 7 panel 2



FIG. 8

## SEQUENCE LISTING

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&lt;141&gt;

&lt;150&gt; SE 9902056-2

&lt;151&gt; 1999-06-03

&lt;160&gt; 2

&lt;170&gt; PatentIn Ver. 2.1

&lt;210&gt; 1

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&lt;222&gt; (91)..(3654)

&lt;400&gt; 1

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gtg gcc tgg gcg ctc agc ctg tgg cca ggg ttc acg gac acc ttc aac 162
Val Ala Trp Ala Leu Ser Leu Trp Pro Gly Phe Thr Asp Thr Phe Asn
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Val Val Gly Ala Pro Leu Glu Thr Asn Gly Tyr Gln Lys Thr Gly Asp
    60      65      70

gtg tac aag tgt cca gtg atc cac ggg aac tgc acc aaa ctc aac ctg 354
Val Tyr Lys Cys Pro Val Ile His Gly Asn Cys Thr Lys Leu Asn Leu
    75      80      85

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 Ile Pro Gly Ser Arg Thr Ala Phe Phe Gly Tyr Thr Val Gln Gln His  
 35 40 45  
 Asp Ile Ser Gly Asn Lys Trp Leu Val Val Gly Ala Pro Leu Glu Thr  
 50 55 60  
 Asn Gly Tyr Gln Lys Thr Gly Asp Val Tyr Lys Cys Pro Val Ile His  
 65 70 75 80

Gly Asn Cys Thr Lys Leu Asn Leu Gly Arg Val Thr Leu Ser Asn Val  
 85 90 95  
 Ser Glu Arg Lys Asp Asn Met Arg Leu Gly Leu Ser Leu Ala Thr Asn  
 100 105 110  
 Pro Lys Asp Asn Ser Phe Leu Ala Cys Ser Pro Leu Trp Ser His Glu  
 115 120 125  
 Cys Gly Ser Ser Tyr Tyr Thr Thr Gly Met Cys Ser Arg Val Asn Ser  
 130 135 140  
 Asn Phe Arg Phe Ser Lys Thr Val Ala Pro Ala Leu Gln Arg Cys Gln  
 145 150 155 160  
 Thr Tyr Met Asp Ile Val Ile Val Leu Asp Gly Ser Asn Ser Ile Tyr  
 165 170 175  
 Pro Trp Val Glu Val Gln His Phe Leu Ile Asn Ile Leu Lys Lys Phe  
 180 185 190  
 Tyr Ile Gly Pro Gly Gln Ile Gln Val Gly Val Val Gln Tyr Gly Glu  
 195 200 205  
 Asp Val Val His Glu Phe His Leu Asn Asp Tyr Arg Ser Val Lys Asp  
 210 215 220  
 Val Val Glu Ala Ala Ser His Ile Glu Gln Arg Gly Gly Thr Glu Thr  
 225 230 235 240  
 Arg Thr Ala Phe Gly Ile Glu Phe Ala Arg Ser Glu Ala Phe Gln Lys  
 245 250 255  
 Gly Gly Arg Lys Gly Ala Lys Lys Val Met Ile Val Ile Thr Asp Gly  
 260 265 270  
 Glu Ser His Asp Ser Pro Asp Leu Glu Lys Val Ile Gln Gln Ser Glu  
 275 280 285  
 Arg Asp Asn Val Thr Arg Tyr Ala Val Ala Val Leu Gly Tyr Tyr Asn  
 290 295 300  
 Arg Arg Gly Ile Asn Pro Glu Thr Phe Leu Asn Glu Ile Lys Tyr Ile  
 305 310 315 320  
 Ala Ser Asp Pro Asp Asp Lys His Phe Phe Asn Val Thr Asp Glu Ala  
 325 330 335  
 Ala Leu Lys Asp Ile Val Asp Ala Leu Gly Asp Arg Ile Phe Ser Leu  
 340 345 350  
 Glu Gly Thr Asn Lys Asn Glu Thr Ser Phe Gly Leu Glu Met Ser Gln  
 355 360 365  
 Thr Gly Phe Ser Ser His Val Val Glu Asp Gly Val Leu Leu Gly Ala  
 370 375 380  
 Val Gly Ala Tyr Asp Trp Asn Gly Ala Val Leu Lys Glu Thr Ser Ala  
 385 390 395 400

Gly Lys Val Ile Pro Leu Arg Glu Ser Tyr Leu Lys Glu Phe Pro Glu  
 405 410 415  
 Glu Leu Lys Asn His Gly Ala Tyr Leu Gly Tyr Thr Val Thr Ser Val  
 420 425 430  
 Val Ser Ser Arg Gln Gly Arg Val Tyr Val Ala Gly Ala Pro Arg Phe  
 435 440 445  
 Asn His Thr Gly Lys Val Ile Leu Phe Thr Met His Asn Asn Arg Ser  
 450 455 460  
 Leu Thr Ile His Gln Ala Met Arg Gly Gln Gln Ile Gly Ser Tyr Phe  
 465 470 475 480  
 Gly Ser Glu Ile Thr Ser Val Asp Ile Asp Gly Asp Gly Val Thr Asp  
 485 490 495  
 Val Leu Leu Val Gly Ala Pro Met Tyr Phe Asn Glu Gly Arg Glu Arg  
 500 505 510  
 Gly Lys Val Tyr Val Tyr Glu Leu Arg Gln Asn Arg Phe Val Tyr Asn  
 515 520 525  
 Gly Thr Leu Lys Asp Ser His Ser Tyr Gln Asn Ala Arg Phe Gly Ser  
 530 535 540  
 Ser Ile Ala Ser Val Arg Asp Leu Asn Gln Asp Ser Tyr Asn Asp Val  
 545 550 555 560  
 Val Val Gly Ala Pro Leu Glu Asp Asn His Ala Gly Ala Ile Tyr Ile  
 565 570 575  
 Phe His Gly Phe Arg Gly Ser Ile Leu Lys Thr Pro Lys Gln Arg Ile  
 580 585 590  
 Thr Ala Ser Glu Leu Ala Thr Gly Leu Gln Tyr Phe Gly Cys Ser Ile  
 595 600 605  
 His Gly Gln Leu Asp Leu Asn Glu Asp Gly Leu Ile Asp Leu Ala Val  
 610 615 620  
 Gly Ala Leu Gly Asn Ala Val Ile Leu Trp Ser Arg Pro Val Val Gln  
 625 630 635 640  
 Ile Asn Ala Ser Leu His Phe Glu Pro Ser Lys Ile Asn Ile Phe His  
 645 650 655  
 Arg Asp Cys Lys Arg Ser Gly Arg Asp Ala Thr Cys Leu Ala Ala Phe  
 660 665 670  
 Leu Cys Phe Thr Pro Ile Phe Leu Ala Pro His Phe Gln Thr Thr Thr  
 675 680 685  
 Val Gly Ile Arg Tyr Asn Ala Thr Met Asp Glu Arg Arg Tyr Thr Pro  
 690 695 700  
 Arg Ala His Leu Asp Glu Gly Gly Asp Arg Phe Thr Asn Arg Ala Val  
 705 710 715 720

Leu Leu Ser Ser Gly Gln Glu Leu Cys Glu Arg Ile Asn Phe His Val  
 725 730 735  
 Leu Asp Thr Ala Asp Tyr Val Lys Pro Val Thr Phe Ser Val Glu Tyr  
 740 745 750  
 Ser Leu Glu Asp Pro Asp His Gly Pro Met Leu Asp Asp Gly Trp Pro  
 755 760 765  
 Thr Thr Leu Arg Val Ser Val Pro Phe Trp Asn Gly Cys Asn Glu Asp  
 770 775 780  
 Glu His Cys Val Pro Asp Leu Val Leu Asp Ala Arg Ser Asp Leu Pro  
 785 790 795 800  
 Thr Ala Met Glu Tyr Cys Gln Arg Val Leu Arg Lys Pro Ala Gln Asp  
 805 810 815  
 Cys Ser Ala Tyr Thr Leu Ser Phe Asp Thr Thr Val Phe Ile Ile Glu  
 820 825 830  
 Ser Thr Arg Gln Arg Val Ala Val Glu Ala Thr Leu Glu Asn Arg Gly  
 835 840 845  
 Glu Asn Ala Tyr Ser Thr Val Leu Asn Ile Ser Gln Ser Ala Asn Leu  
 850 855 860  
 Gln Phe Ala Ser Leu Ile Gln Lys Glu Asp Ser Asp Gly Ser Ile Glu  
 865 870 875 880  
 Cys Val Asn Glu Glu Arg Arg Leu Gln Lys Gln Val Cys Asn Val Ser  
 885 890 895  
 Tyr Pro Phe Phe Arg Ala Lys Ala Lys Val Ala Phe Arg Leu Asp Ser  
 900 905 910  
 Glu Phe Ser Lys Ser Ile Phe Leu His His Leu Glu Ile Glu Leu Ala  
 915 920 925  
 Ala Gly Ser Asp Ser Asn Glu Arg Asp Ser Thr Lys Glu Asp Asn Val  
 930 935 940  
 Ala Pro Leu Arg Phe His Leu Lys Tyr Glu Ala Asp Val Leu Phe Thr  
 945 950 955 960  
 Arg Ser Ser Ser Leu Ser His Tyr Glu Val Lys Leu Asn Ser Ser Leu  
 965 970 975  
 Glu Arg Tyr Asp Gly Ile Gly Pro Pro Phe Ser Cys Ile Phe Arg Ile  
 980 985 990  
 Gln Asn Leu Gly Leu Phe Pro Ile His Gly Met Met Met Lys Ile Thr  
 995 1000 1005  
 Ile Pro Ile Ala Thr Arg Ser Gly Asn Arg Leu Leu Lys Leu Arg Asp  
 1010 1015 1020  
 Phe Leu Thr Asp Glu Ala Asn Thr Ser Cys Asn Ile Trp Gly Asn Ser  
 1025 1030 1035 1040

Thr Glu Tyr Arg Pro Thr Pro Val Glu Glu Asp Leu Arg Arg Ala Pro  
1045 1050 1055

Gln Leu Asn His Ser Asn Ser Asp Val Val Ser Ile Asn Cys Asn Ile  
1060 1065 1070

Arg Leu Val Pro Asn Gln Glu Ile Asn Phe His Leu Leu Gly Asn Leu  
1075 1080 1085

Trp Leu Arg Ser Leu Lys Ala Leu Lys Tyr Lys Ser Met Lys Ile Met  
1090 1095 1100

Val Asn Ala Ala Leu Gln Arg Gln Phe His Ser Pro Phe Ile Phe Arg  
1105 1110 1115 1120

Glu Glu Asp Pro Ser Arg Gln Ile Glu Phe Glu Ile Ser Lys Gln Glu  
1125 1130 1135

Asp Trp Gln Val Pro Ile Trp Ile Ile Val Gly Ser Thr Leu Gly Gly  
1140 1145 1150

Leu Leu Leu Leu Ala Leu Leu Val Leu Ala Leu Arg Lys Leu Gly Phe  
1155 1160 1165

Phe Arg Ser Ala Arg Arg Arg Arg Glu Pro Gly Leu Asp Pro Thr Pro  
1170 1175 1180

Lys Val Leu Glu  
1185



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 00/01135

## A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07K 14/705, A61K 38/17, C07K 16/28

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Int. J. Cancer, Volume 66, 1996, Michele Genini et al, "Isolation of genes differentially expressed in human primary myoblasts and embryonal rhabdomyosarcoma" page 571 - page 577	6-9,11,22, 26-27,30-44, 48-49,71-85, 89-90
A	--	1-5,10, 12-21,23-25, 28-29,45-47, 50-70,86-
X	Developmental Dynamics, Volume 204, 1995, Donald Gullberg et al, "Up-Regulation of a Novel Integrin alpha-Chain (alpha mt) on Human Fetal Myotubes" page 57 - page 65	5
A	--	1-4,6-105

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier document but published on or after the international filing date

"C" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

20 Sept 2000

Date of mailing of the international search report

02-10-2000

Name and mailing address of the ISA

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 00/01135

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9219647 A1 (THE SCRIPPS RESEARCH INSTITUTE), 12 November 1992 (12.11.92)	1-21,30-44, 48-65,69-85, 89-93,95, 97-105
A	--	22,26-29
X	J Biol Chem., Volume 273, No 32, August 1998, Lisbet Camper et al, "Isolation, Cloning, and Sequence Analysis of the Integrin Subunit alpha 10, a beta1-associated Collagen Binding Integrin Expressed on Chondrocytes", page 20383 - page 20389	1-21,30-44, 48-65,69-85, 89-93,97-105
A	--	22,26-29
X	File WPI, Derwent accession no. 1997-297879, ATHENA NEUROSCIENCES INC: "Uses of humanised alpha-4 integrin antibody - for treatment of asthma, atherosclerosis, AIDS, dementia, etc."; & WO,A1,9718838, 19970529 DW199727	1-21,29-44, 48-65,69-70, 91-93, 96-100,103,
A	--	
A	WO 9822500 A2 (COR THERAPEUTICS, INC.), 28 May 1998 (28.05.98)	1-105
A	--	
A	Frontiers in Bioscience, Volume 3, October 1998, Donald Gullberg et al, "Integrins during muscle development and in muscular dystrophies" page 1039 - page 1050	1-105
P,X	--	
P,X	THE JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 274, No 36, Sept 1999, Teet Velling et al, "cDNA Cloning and Chromosomal Localization of Human alpha11 Integrin", page 25735 - page 25742	1-105
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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 00/01135

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>National Library of Medicine (NLM), file Medline,  Medline accession no. 99417678, Lehnert K et al:  "Cloning, sequence analysis, and chromosomal  localization of the novel human integrin alpha1  subunit (ITGA11);  &amp; Genomics 1999 Sep 1; 60 (2): 179-87  &amp; GenBank AF 109681</p> <p style="text-align: center;">--  -----</p>	1-105

## INTERNATIONAL SEARCH REPORT

International application No.  
**PCT/SE00/01135****Box I** Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: **2-4, 30-40, 44-61, 65-81, 85-90, 96, 97, 101-103 partially and**  
because they relate to subject matter not required to be searched by this Authority, namely:  
**42-43, 63-64, 83-84, 95 and 104-105 completely.**

**See extra sheet\***

2. ☒ Claims Nos.: **12, 21, 29, 50-70, 91 and 96 (partially)**  
because they relate to parts of the international application that do not comply with the prescribed requirements to such  
an extent that no meaningful international search can be carried out, specifically:

**See extra sheet\*\***

3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

**Box II** Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is  
restricted to the invention first mentioned in the claims: it is covered by claims Nos.:

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/SE00/01135

\* claims 2-4, 30-40, 44-61, 65-81, 85-90, 96, 97, 101-103 partially and 42-43, 63-64, 83-84, 95 and 104-105 completely

Claims 2-4, 30-40, 44-61, 65-81, 85-90, 96, 97, 101-103 partially and 42-43, 63-64, 83-84, 95 and 104-105 completely relate to methods of treatment of the human or animal body by therapy or diagnostic methods practised on the human or animal body. See PCT Rule. 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds.

\*\* claims 12, 21, 29, 50-70, 91 and 96 (partially) relates to a binding entity specific to  $\alpha 11$  integrin or homologues or fragments thereof. The wording "binding entities" is too broad to permit a meaningful search, i.e. claims directed to these entities fails to comply with PCT-Art . The search is limited to antibodies directed towards  $\alpha 11$  integrins.

## 01/08/00

PCT/SE 00/01135

Patent document cited in search report:	Publication date	Patent family member(s)	Publication date
WO 9219647 A1	12/11/92	AU 1896392 A	21/12/92
		US 5310874 A	10/05/94
		US 5589570 A	31/12/96
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WO 9822500 A2	28/05/98	AU 5595198 A	10/06/98
		EP 0942931 A	22/09/99